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(FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002

```
L1         45382 S ALPHA (W) AMYLASE?
L2         12942 S ASPERGILLUS(W)ORYZAE
L3          1829 S L1 AND L2
L4        404497 S THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC
L5            35 S L3 AND L4
L6            27 DUP REM L5 (8 DUPLICATES REMOVED)
L7            73 S FUNGAMYL
L8             2 S L6 AND L7
L9         78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER
L10            2 S L5 AND L9
L11       330343 S IMMOBILI?
L12            5 S L5 AND L11
                 E BISGARD-FRANTZEN H/AU
L13            2 S E4
                 E AVENDSEN A/AU
                 E SVENDSEN A/AU
L14          286 S E3
L15            5 S L3 AND L14
L16            3 DUP REM L15 (2 DUPLICATES REMOVED)
                 E PEDERSEN S/AU
L17         1236 S E3
L18            1 S L17 AND L5
L19            1 S L3 AND L17
L20         1524 S L12 OR L14 OR L17
L21           29 S L20 AND L11
L22           20 DUP REM L21 (9 DUPLICATES REMOVED)
                 E CHRISTENSEN T/AU
L23         1060 S E3
L24            1 S L23 AND SUBTILISIN
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Fulat # 5528338

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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Jan 25	BLAST(R) searching in REGISTRY available in STN on the Web
NEWS 3	Jan 29	FSTA has been reloaded and moves to weekly updates
NEWS 4	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency
NEWS 5	Feb 19	Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6	Mar 08	Gene Names now available in BIOSIS
NEWS 7	Mar 22	TOXLIT no longer available
NEWS 8	Mar 22	TRCTHERMO no longer available
NEWS 9	Mar 28	US Provisional Priorities searched with P in CA/CAplus and USPATFULL
NEWS 10	Mar 28	LIPINSKI/CALC added for property searching in REGISTRY
NEWS 11	Apr 02	PAPERCHEM no longer available on STN. Use PAPERCHEM2 instead.
NEWS 12	Apr 08	"Ask CAS" for self-help around the clock
NEWS 13	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 14	Apr 09	ZDB will be removed from STN
NEWS 15	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 16	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 17	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 18	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 19	Jun 03	New e-mail delivery for search results now available
NEWS 20	Jun 10	MEDLINE Reload
NEWS 21	Jun 10	PCTFULL has been reloaded

NEWS EXPRESS	February 1 CURRENT WINDOWS VERSION IS V6.0d, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 05 FEBRUARY 2002
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=> s alpha (w) amylase?
L1 45382 ALPHA (W) AMYLASE?

=> s aspergillus(w)oryzae
L2 12942 ASPERGILLUS(W) ORYZAE

=> s l1 and l2
L3 1829 L1 AND L2

=> s themostab? or "acid-resistant" or acidic
L4 404497 THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC

=> s l3 and l4
L5 35 L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 27 DUP REM L5 (8 DUPLICATES REMOVED)

=> d 1-27 ibib ab

L6 ANSWER 1 OF 27 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of Fungamyl-like **alpha-amylase**
, useful for production of maltose syrups, includes mutations
that improve stability against heat and **acidic** pH;
plasmid pTAKA17 expression in bacterium cell for syrup
production, dough improvement, brewing and starch
liquefaction
AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE: Novozymes
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]
AB A variant (A) of a Fungamyl-like **alpha-amylase**

(EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of Fungamyl-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L6 ANSWER 2 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:360158 HCAPLUS
DOCUMENT NUMBER: 134:363353
TITLE: Fungamyl-like **Aspergillus oryzae** . **alpha.-amylase** variants with improved thermal stability and applications to starch processes
INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan; Pedersen, Sven
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034784	A1	20010517	WO 2000-DK626	20001110

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 1999-1617 A 19991110
AB The invention relates to a variant of a parent Fungamyl-like fungal . **alpha.-amylase**, which exhibits improved thermal stability at **acidic** pH suitable for, e.g., starch processes. Cloning, amino acid and encoding nucleotide sequences, and mutagenesis of **.alpha.-amylase** from **Aspergillus oryzae** are provided. Construction of variant Q153S .**alpha** **.-amylase** (Q173S pre-.**alpha.-amylase**) from A. oryzae is disclosed.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:930695 HCAPLUS
DOCUMENT NUMBER: 136:84756
TITLE: High-quality Japanese sake brewing by the newly developed enzyme preparation "Gluc Gin"
AUTHOR(S): Iwano, Kimio; Amano, Hitoshi

CORPORATE SOURCE: Akita Prefect. Univ., Nakano Shimo-shinjyo, Akita, 010-0195, Japan

SOURCE: Nippon Jozo Kyokaishi (2001), 96(11), 789-795
CODEN: NJKYES; ISSN: 0914-7314

PUBLISHER: Nippon Jozo Kyokai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

AB A new enzyme prepn. "Gluc Gin" which can be used for brewing high-quality Japanese sake (Junmaiginjou-shu, ginjou-shu, or junnmai-shu etc.) at low-temp. fermn., has been developed. The brewing using "Gluc Gin" was performed at 13 Japanese sake breweries. "Gluc Gin" was used as a replacement of tome-koji, or as an addn. to tome-koji at the test brewing. Results showed that fermn. (moromi) periods, the amt. of pure alc. acquisition, and the ratio of the sake cake of "Gluc Gin" differed only slightly compared with those of the control with no enzyme addn. No significant differences regarding the compn. of the general components, amino acid, or the fragrance components between the test sake and control sake were recognized. It was clearly shown that newly developed "Gluc Gin" developed functions almost equally to Ginjou-koji, and can be used as a replacement for tome-koji in low-temp. fermn.

L6 ANSWER 4 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:570968 HCAPLUS
DOCUMENT NUMBER: 136:117535
TITLE: Effects of raw materials and various molds on the production of koji
AUTHOR(S): Yi, Sang-Duk; Yang, Jae-Seung; Lee, Gyu-Hee; Choi, Seong-Hyun; Oh, Man-Jin
CORPORATE SOURCE: Detection Lab. of Irradiated Food, Korea Atomic Energy Research Institute, Taejon, 305-353, S. Korea
SOURCE: Journal of Food Science and Nutrition (2001), 6(2), 101-106
CODEN: JFSNFW; ISSN: 1226-332X
PUBLISHER: Korean Society of Food Science and Nutrition
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **.alpha.-Amylase** and glucoamylase activities were higher in koji with 40% water vs. 30 and 50% water, and A. oryzae exhibited very high **.alpha.-amylase** and glucoamylase activities compared to A. sojae and A. niger. **Acidic**, neutral and alk. protease activities also showed higher activities in koji prepd. with flour, Korean wheat powder and soybean powder with 40% water based on the wt. of the sample. **.alpha.-Amylase**, glucoamylase, and **acidic**, neutral and alk. protease activities of all the koji samples increased up to 3-4 days of incubation and maintained nearly the same level or slightly decreased after 5 days of incubation. The protease activities of A. oryzae and A. sojae showed nearly the same trend regardless of differences in substrate conditions and koji materials, but those of A. niger showed a lower activity than those of A. oryzae and A. sojae. These results suggest that the prepn. of koji is possible with Korean wheat powder and soybean powder and A. sojae can be utilized as a new strain for fermented foods using soybean as the main materials to increase functional properties and produce products having a new taste and flavor.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

DUPLICATE 1

L6 ANSWER 5 OF 27 MEDLINE
ACCESSION NUMBER: 2001082941 MEDLINE
DOCUMENT NUMBER: 20472909 PubMed ID: 11015693
TITLE: Release characteristics of a short-chain fatty acid, n-butyric acid, from its beta-cyclodextrin ester conjugate

in rat biological media.

AUTHOR: Hirayama F; Ogata T; Yano H; Arima H; Udo K; Takano M; Uekama K

CORPORATE SOURCE: Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan.

SOURCE: JOURNAL OF PHARMACEUTICAL SCIENCES, (2000 Nov) 89 (11) 1486-95.
Journal code: 2985195R. ISSN: 0022-3549.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010105

AB 6(A)-O-(n-Butanoyl)-beta-cyclodextrin was prepared and its hydrolysis behavior in aqueous solutions and in rat intestinal fluids was investigated. Furthermore, the enzymatic hydrolyses of the n-butyric acid-beta-cyclodextrin conjugate using **alpha-amylase** and esterase were studied to gain insight into the release behavior of n-butyric acid from the conjugate. The hydrolysis of the conjugate proceeded according to a first-order kinetics in aqueous solution, and gave a V-shaped pH profile, indicating a specific acid-base-catalyzed hydrolysis at **acidic** and neutral-alkaline regions, respectively. The half-lives (t(1/2)) of the conjugate at pH 4.4, 6.8, and 7.4 at 37 degrees C were approximately 580, 43, and 6 days, respectively, indicating that the conjugate is stable in aqueous solution. No appreciable release of n-butyric acid from the conjugate was observed in the stomach and small intestinal contents of rats, or in the small and large intestinal homogenates of rats. On the other hand, a fast disappearance of the conjugate and an appearance of n-butyric acid were observed in the cecal and colonic contents of rats. The t(1/2) values of the disappearance were approximately 4, 1, and 6 h in 10 and 15% cecal contents and 10% colonic contents, respectively, and the appearance of n-butyric acid after 6 h was approximately 10% in the 15% cecal contents. **Aspergillus oryzae alpha-amylase** hydrolyzed the conjugate to small saccharide conjugates, such as the triose and maltose conjugates, but there was no appreciable release of n-butyric acid. The conjugate was less susceptible to carboxylic esterase (from porcine live), thus releasing no appreciable amounts of n-butyric acid. On the other hand, a fast release of n-butyric acid was observed when the esterase was employed after amylase hydrolysis, suggesting that two types of enzymes, sugar-degrading and ester-hydrolyzing enzymes, are necessary for the release of n-butyric acid from the conjugate in large intestinal contents.
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L6 ANSWER 6 OF 27 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:619935 HCAPLUS
DOCUMENT NUMBER: 127:261912
TITLE: Preparation of low salt and functional Kochujang containing chitosan

AUTHOR(S): Na, Sang-Eon; Seo, Kyu-Seok; Choi, Jung-Ho; Song, Geun-Seoub; Choi, Dong-Seong

CORPORATE SOURCE: Div. Food Analysis, Public Health Environment Inst. Chollabukdo, Jeonju, 560-200, S. Korea

SOURCE: Han'guk Sikp'um Yongyang Hakhoechi (1997), 10(2), 193-200
CODEN: HGSHFX; ISSN: 1225-4339

PUBLISHER: Korean Society of Food and Nutrition
DOCUMENT TYPE: Journal
LANGUAGE: Korean

AB In order to manuf. low-salt and functional Kochujang (red pepper spice paste made with the addn. of wheat flour, glutinous rice, and Koji enzyme from **Aspergillus oryzae**), the salt amt. was reduced from 9 to 6% and chitosan was added at 0.1 to 0.25%. The contents of ash, moisture, crude fat and crude protein in Kochujang were not affected by the reduced salt concn. and chitosan addn. The pH and titrn. acidity were not significantly changed by the addn. of chitosan. The ethanol content was higher in the 6% salt Kochujang than in the 9% salt Kochujang and decreased with the addn. of chitosan. The reducing sugar content was lower in the 6% salt Kochujang than in the 9% salt Kochujang and increased with the chitosan addn. The **.alpha.-amylase** activity was slightly inhibited by the addn. of chitosan, but .beta.-amylase, **acidic** protease, and neutral protease activities were not affected. Amino group nitrogen and ammonia nitrogen contents were higher in the 6% salt Kochujang than in the 9% salt Kochujang, but the ammonia nitrogen prodn. was decreased by the chitosan addn. The growth of bacteria and yeasts were slightly inhibited by the addn. of chitosan. Thus, the addn. of 0.25% chitosan is suitable in the prepn. of low-salt functional Kochujang.

L6 ANSWER 7 OF 27 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1997-10559 BIOTECHDS
TITLE: A strain of the fungus **Aspergillus oryzae**, a producer of a complex of **acidic** and weakly **acidic** proteases, amylolytic and cytolytic enzymes; e.g. **alpha-amylase**, cellobiohydrolase, cytase and endo-1,4-beta-D-xylanase
AUTHOR: Martynenko L D; Shepelev A P
PATENT ASSIGNEE: Immunogen
PATENT INFO: RU 2070923 27 Dec 1996
APPLICATION INFO: RU 1992-5043065 21 May 1992
PRIORITY INFO: SU 1992-5043065 21 May 1992
DOCUMENT TYPE: Patent
LANGUAGE: Russian
OTHER SOURCE: WPI: 1997-362013 [33]
AB A new strain, **Aspergillus oryzae** 387 (VKPM F-683), produces a complex of **acidic** and weakly **acidic** proteases, amylolytic and cytolytic enzymes, including **alpha-amylase** (EC-3.2.1.1), cellobiohydrolase (EC-3.2.1.91), cytase and endo-1,4-beta-D-xylanase (EC-3.2.1.8). The strain may be useful in the microbiological industries for the production of enzyme preparations for the hydrolysis of plant, animal and microbial substrates, for the production of amino acid mixtures in the food and agricultural industries, and in the fermentation of yeast. In an example, A. oryzae was cultured in a nutrient medium containing 3% barley meal, 3% bran, 1.5% KH2PO4 and tap water on a shaker at 28-30 deg for 42 hr. (6pp)

L6 ANSWER 8 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:485479 HCAPLUS
DOCUMENT NUMBER: 125:216568
TITLE: A study on a Chinese fermented food "Jinhua Huotui". II. Identification and enzyme activity of molds isolated from Chinese fermented food "Jinhua Huotui"
AUTHOR(S): Wagu, Yutaka; Kakuta, Toshitaka; Shindo, Hitoshi; Koizumi, Takeo
CORPORATE SOURCE: Bio'c Co., Ltd., Toyohashi, 441, Japan
SOURCE: Nippon Shokuhin Kagaku Kogaku Kaishi (1996), 43(7), 796-805
CODEN: NSKKEF; ISSN: 1341-027X
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB Molds from "jinhua Huotui", a traditional fermented food produced in

China, were identified and their enzyme activities were evaluated. All strains isolated were identified as being of the genus Penicillium (30 strains) and of the genus Aspergillus (25 strains). Of the genus Penicillium, P. aurantiograseum, P. solitum, P. implicatum, P. viridicatum, P. fellutanum and other four kinds of molds were identified. Of the genes Aspergillus, A. versicolor, A. sydowii, A. candidus, Eurotium rubrum, E. amstelodami and other three kinds of molds were identified. As to enzyme activities, the genus Penicillium showed strong **acidic** protease activity, and the genus Aspergillus showed strong neutral and alk. protease activities. Esp., A. ochraceus had strong protease activity as compared to the std. strain of A. oryzae. The genus Penicillium showed stronger lipase activities than the genus Aspergillus. Esp., P. fellutanum, P. canescens had very strong lipase activities. As to . **alpha.-amylase** and phosphatase activities, all molds isolated from "jinhua Huotui" showed low activities. These results indicate that the genus Aspergillus plays the main role for decompn. of protein, and the genus Penicillium does for decompn. of lipid in "jinhua Huotui".

L6 ANSWER 9 OF 27 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 2
ACCESSION NUMBER: 95:70384 SCISEARCH
THE GENUINE ARTICLE: QB740
TITLE: PURIFICATION AND PARTIAL CHARACTERIZATION OF 2 PROTEINACEOUS **ALPHA-AMYLASE** INHIBITORS FROM TRITICALE
AUTHOR: IDA E I; FINARDIFILHO F; LAJOLO F M (Reprint)
CORPORATE SOURCE: UNIV SAO PAULO, FAC CIENCIAS FARMACEUT, DEPT ALIMENTOS & NUTR EXPTL, C POSTAL 66083, BR-05389 SAO PAULO, SP, BRAZIL (Reprint); UNIV SAO PAULO, FAC CIENCIAS FARMACEUT, DEPT ALIMENTOS & NUTR EXPTL, C POSTAL 66083, BR-05389 SAO PAULO, SP, BRAZIL; UNIV ESTAD LONDRINA, DEPT TECNOL ALIMENTOS & MEDICAMENTOS, BR-86051 LONDRINA, PARANA, BRAZIL
COUNTRY OF AUTHOR: BRAZIL
SOURCE: JOURNAL OF FOOD BIOCHEMISTRY, (1994) Vol. 18, No. 2, pp. 83-102.
ISSN: 0145-8884.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 41
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A total of six **alpha-amylase** inhibitory proteins (isoinhibitors) were extracted from triticale (Triticum x Secale) seeds and two of them were purified to homogeneity. The isoinhibitors were extracted by 70% ethanol and produced, by Sephadex G-100 chromatography, two peaks that exhibited **alpha-amylase** inhibitory activity. Further purification of the most active peak by DEAE-cellulose chromatography resulted in six active fractions. Two of them designated as TAI-5 and TAI-6, considered to be homogeneous by both **acidic** and alkaline electrophoresis, were partially characterized. The isoelectric points were 4.80 and 4.70, and the molecular weights 39,200 and 29,200, respectively. Under dissociating conditions the molecular weights were 13,500 and 13,000, suggesting that the isoinhibitors are composed of three and two subunits, respectively. Both isoinhibitors were stable at different pHs, relatively stable at 98C, and resistant to proteolysis by trypsin, chymotrypsin and pepsin. The optimum interaction pH for both isoinhibitors with human salivary amylase was 7.9. They exhibited specificity to human salivary and porcine pancreatic **alpha-amylases**, but had no inhibitory activity on Bacillus subtillis, **Aspergillus oryzae** and endogenous triticale **alpha-amylases**.

L6 ANSWER 10 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:242982 HCAPLUS
DOCUMENT NUMBER: 120:242982
TITLE: Microflora and enzyme activities of Chinese Qu
AUTHOR(S): Yokoyama, Naoyuki; Tanaka, Kazuyoshi; Du, Lianxing; Aramaki, Isao; Kizaki, Yasuzo; Kobayashi, Shinya; Okazaki, Naoto
CORPORATE SOURCE: Natl. Res. Inst. Brew., Tokyo, 114, Japan
SOURCE: Nippon Jozo Kyokaishi (1994), 89(1), 72-6
CODEN: NJKYES; ISSN: 0914-7314
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB Microflora, enzyme activities, and components of Chinese Qu, which has been used as the starter for the traditional Chinese liquor making, were studied. There were several layers on a cutting section of Qu. Fungi (except yeast) were present in large nos. in the 2nd and 3rd layers, but only a few in the 1st (surface) and center layers. Yeasts were present in large nos. in the 2nd layer. Enzyme activities were the highest in the 4th layer. **.alpha.-Amylase** activity was lower, glucoamylase activity was slightly lower, acid-proteinase activity was the same, and acid-carboxypeptidase activity was lower in the Qu than those of koji made by **Aspergillus oryzae**. The content of lactic acid in Qu was the largest in the 1st and 2nd layers and reached 1-2 wt.% of Qu. The EtOH content was the largest in the 2nd layer, in agreement with the distribution of the organisms. Many Absidia were identified from Qu contrary to former reports that fungi sepd. in Qu were mainly Rhizopus. The same microbial layer construction as the Chinese Qu reappeared in exptl. Qu-making.

L6 ANSWER 11 OF 27 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 3
ACCESSION NUMBER: 91020536 EMBASE
DOCUMENT NUMBER: 1991020536
TITLE: Transformation of Trichoderma viride using the Neurospora crassa pyr4 gene and its use in the expression of a Taka-amylase A gene from **Aspergillus oryzae**.
AUTHOR: Cheng C.; Tsukagoshi N.; Udaka S.
CORPORATE SOURCE: Faculty of Agriculture, Nagoya University,Nagoya 464, Japan
SOURCE: Current Genetics, (1990) 18/5 (453-456).
ISSN: 0172-8083 CODEN: CUGED5
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A pyrG mutant of Trichoderma viride, a very efficient cellulase producer, was isolated from among 5-fluoroorotic **acid-resistant** mutants. The mutation was complemented with the pyr4 gene of Neurospora crassa and used as a selection marker for the transformation of T. viride. A plasmid vector, pDJB1-Taa, carrying both the pyr4 gene and a gene encoding Taka-amylase A from **Aspergillus oryzae**, was constructed and introduced into protoplasts of T. viride pyrG-. The transformation frequency was 1-10 transformants (3 on average) per .mu.g DNA. One transformant showed highly elevated **.alpha.-amylase** production (about 17 times higher than the recipient level) and the integration of more than one copy of the Taka-amylase gene.

L6 ANSWER 12 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1988:374253 BIOSIS
DOCUMENT NUMBER: BA86:58163
TITLE: MIRIN-MAKING AT LOW ALCOHOL CONCENTRATION WITH KOJI

PREPARED WITH A NEW MUTANT OF ASPERGILLUS-USAMII.
AUTHOR(S): OYASHIKI H; UCHIDA M; OBAYASHI A; OKA S
CORPORATE SOURCE: CENT. RES. LAB., TAKARA SHUZO CO. LTD., SETA 3-4-1, OTSU-SHI, SHIGA 520-21, JPN.
SOURCE: J FERMENT TECHNOL, (1988) 66 (3), 333-340.
CODEN: JFTED8. ISSN: 0385-6380.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB We examined the productivity of mirin-making and the quality of the mirin produced using koji prepared with a new mutant (mutant CA) that originated from Aspergillus usamii mut. shiro-usamii. The koji prepared with the mutant CA contained a large amount of citric acid. Therefore, the concentration of the brewing alcohol added to prevent bacterial contamination of the mash was decreased to 6.0% from the 12.5% needed when the mash was made with koji of the conventional **Aspergillus oryzae**. A mash containing this low concentration of alcohol was incubated with koji of the mutant CA and enzyme preparations such as **alpha.-amylase** (6,000 DU/kg mash) from Bacillus subtilis and **acidic** protease (1,000 PU/kg mash) from Aspergillus niger. The starch and protein in this mash was sufficiently digested. The yield of mirin obtained from this mash was high (96% based on the mash weight), and the resulting mirin contained much citric acid, malic acid, succinic acid, nitrogen compounds, isomaltose, isomaltotriose, and oligosaccharides. The taste of the mirin was refreshingly sour and flavorsome.

L6 ANSWER 13 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1988:264315 BIOSIS
DOCUMENT NUMBER: BA86:3559
TITLE: USE OF KOJI PREPARED WITH A HIGH CITRIC ACID PRODUCING MUTANT OF ASPERGILLUS-USAMII AS A RAW MATERIAL FOR SAKE BREWING.
AUTHOR(S): OYASHIKI H; MURATA K; HIRAI N; KUROSE N; UCHIDA M; OBAYASHI A; OKA S
CORPORATE SOURCE: CENTRAL RES. LAB., TAKARA SHUZO CO. LTD., SETA 3-4-1, OTSU-SHI, SHIGA 520-21.
SOURCE: J FERMENT TECHNOL, (1988) 66 (1), 111-116.
CODEN: JFTED8. ISSN: 0385-6380.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB There is a possibility of developing a new kind of sake in which the refreshing sour taste of citric acid is introduced. In this study, we bred a mutant of Aspergillus usamii mut, shiro-usamii that produced much citric acid. The koji prepared with the mutant contained about 20 mg of citric acid per gram of dry koji, twice that of the koji of the parental strain. The activities of **.alpha.-amylase**, glucoamylase, and **acidic** protease in the koji prepared with the mutant were 82%, 94%, and 95%, respectively, those of the parental strain. Using this koji with the mutant, sake was produced. The levels of citric acid and isoamyl acetate was 5.1 and 1.4 times, respectively, those of sake prepared with koji of A. oryzae. Sensory tests indicated that sake made with koji with the mutant was refreshingly sour, with a good aroma.

L6 ANSWER 14 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:34075 HCAPLUS
DOCUMENT NUMBER: 108:34075
TITLE: Studies on the active site of taka-amylase A: its action on phenyl maltooligosides with a charge at their non-reducing-ends
AUTHOR(S): Nagamine, Yuko; Sumikawa, Michito; Omichi, Kaoru; Ikenaka, Tokuji
CORPORATE SOURCE: Coll. Sci., Osaka Univ., Osaka, 560, Japan

SOURCE: J. Biochem. (Tokyo) (1987), 102(4), 767-75
CODEN: JOBIAO; ISSN: 0021-924X

DOCUMENT TYPE: Journal
LANGUAGE: English

AB Five modified maltooligosaccharides, Ph O-6-amino-6-deoxy-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-.alpha.-D-glucopyranoside (AG4P), Ph O-(.alpha.-D-glucopyranosyluronic acid)-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-.alpha.-D-glucopyranoside (CG4P), Ph O-6-amino-6-deoxy-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-.alpha.-D-glucopyranoside (AG5P), Ph O-(.alpha.-D-glucopyranosyluronic acid)-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-.alpha.-D-glucopyranoside (CG5P), and Ph O-6-deoxy-6-[(2-pyridyl)amino]-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-O-.alpha.-D-glucopyranosyl-(1.fwdarw.4)-.alpha.-D-glucopyranoside (FG4P), were prepd. to examine the active site of Taka-amylase A (TAA) (EC 3.2.1.1, from **Aspergillus oryzae**). Ph .alpha.-maltotetraoside (G4P) was predominantly hydrolyzed by TAA to maltose and Ph .alpha.-maltoside (G2P), whereas G2P, Ph .alpha.-glucoside (GP), and PhOH were liberated from AG4P in the ratio of 7:63:30. G4P, Ph .alpha.-maltotrioside (G3P), G2P, and GP were liberated from G5P in the ratio of 1:20:73:6, but AG5P was almost completely hydrolyzed to modified maltotriose and G2P. On the hydrolysis of CG4P and CG5P, no remarkable change was obsd. except for a decrease in the relative reaction rates compared with G4P and G5P, resp. When FG4P and G4P were hydrolyzed in the pH range of 4.5-6.0, the molar ratio of the hydrolysis products of G4P remained almost const. However, the hydrolysis of FG4P changed with pH, i.e., GP was predominantly formed at lower pHs, whereas the formation of G2P increased at higher pHs. These results suggest the presence of **acidic** amino acids at subsites S3 and S4 in the active site of TAA that interact with amino or pyridylamino groups of the substrates.

L6 ANSWER 15 OF 27 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1986-09735 BIOTECHDS
TITLE: Immobilization of **alpha-amylase** on polymer support;
by passing **acidic alpha-amylase** solution over polyacrylonitrile column and washing out residue at same pH

PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 233589 5 Mar 1986
APPLICATION INFO: DD 1984-272358 28 Dec 1984
PRIORITY INFO: DD 1984-272358 28 Dec 1984
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1986-169840 [27]

AB For immobilization of **alpha-amylase** (EC-3.2.1.1) on a polymer support, the enzyme solution, of pH 2.5-4.5, is passed over polyacrylonitrile powder packed in a chromatography column. The residual constituents of the solution are then removed at the same pH. The enzyme is obtained from fungal mycelium, e.g. that of **Aspergillus oryzae**. It can be fixed to the support from non-purified solutions, without using chemical reactions. The support can be regenerated, charged repeatedly with **alpha-amylase** and re-used for enzymatic reaction. The column is used for enzymatic reactions, e.g. starch saccharification at pH 2.5-4.5. With falling activity of the column, the **alpha-amylase** is removed by washing at pH 7.5-9 and the support is cleaned. The column can be

re-charged with **alpha-amylase** after washing out the alkaline solution. The immobilized **alpha-amylase** is used in the sugar industry. (2pp)

L6 ANSWER 16 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1985:410715 BIOSIS
DOCUMENT NUMBER: BA80:80707
TITLE: ISOLATION OF AN AMYLASE INHIBITOR FROM SETARIA-ITALICA GRAINS BY AFFINITY CHROMATOGRAPHY ON BLUE-SEPHAROSE AND ITS CHARACTERIZATION.
AUTHOR(S): NAGARAJ R H; PATTABIRAMAN T N
CORPORATE SOURCE: DEPARTMENT OF BIOCHEMISTRY, KASTURBA MEDICAL COLLEGE, MANIPAL-576 119, INDIA.
SOURCE: J AGRIC FOOD CHEM, (1985) 33 (4), 646-650.
CODEN: JAFCAU. ISSN: 0021-8561.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB An .**alpha**.-**amylase** inhibitor from S. italica grains was purified 150-fold by chromatography on Blue-Sepharose after neutralization of the acid extract and ammonium sulfate fractionation. The inhibitor was found to be homogenous by polyacrylamide gel electrophoresis [PAGE] and gel chromatography on BioGel P-30. The molecular weight was found to be 24 K. SDS[sodium dedecyl sulfate]-AGE showed that it is made up of two dissimilar polypeptides. Affinity chromatography on immobilized porcine pancreatic amylase and analysis showed that both the polypeptides are essential for the action of the inhibitor. The setaria inhibitor acted on human salivary amylase, human pancreatic amylase, and porcine pancreatic amylase, but had no action on Bacillus subtilis and **Aspergillus oryzae** amylases. It was labile to heat and to extreme **acidic** and alkaline conditions. Pronase, pepsin, trypsin, and .alpha.-chymotrypsin inactivated the inhibitor. Amino groups and guanido groups were found to be essential for its action.

L6 ANSWER 17 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
ACCESSION NUMBER: 1986:112249 BIOSIS
DOCUMENT NUMBER: BA81:22665
TITLE: PURIFICATION AND PROPERTIES OF AN **ALPHA AMYLASE** INHIBITOR SPECIFIC FOR HUMAN PANCREATIC AMYLASE FROM PROSO PANICUM-MILIACEUM SEEDS.
AUTHOR(S): NAGARAJ R H; PATTABIRAMAN T N
CORPORATE SOURCE: DEPARTMENT OF BIOCHEMISTRY, KASTURBA MEDICAL COLLEGE, MANIPAL 576 119, INDIA.
SOURCE: J BIOSCI (BANGALORE), (1985) 7 (3-4), 257-268.
CODEN: JOBSDN. ISSN: 0250-4774.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB An .**alpha**.-**amylase** inhibitor was purified to homogeneity by acid extraction, ammonium sulphate fractionation, chromatography on carboxymethyl-cellulose, diethylaminoethyl-cellulose and Sephadex G-100 from proso grains (Panicum miliaceum). The calculated molecular weight was 14,000. The inhibitor was fairly heat stable under **acidic** and neutral conditions. The factor was more effective by two orders of magnitude in its action on human pancreatic amylase than on human salivary amylase, It did not inhibit on **Aspergillus oryzae**, Bacillus subtilis and porcine pancreatic amylases. Pepsin rapidly inactivated the inhibitor. Chemical modification studies revealed that amino and guanido groups are essential for the action of the inhibitor. The inhibitor was found to protect both human salivary and pancreatic amylases against inactivation by acid. The mode of inhibition was found to be uncompetitive.

L6 ANSWER 18 OF 27 LIFESCI COPYRIGHT 2002 CSA
ACCESSION NUMBER: 83:67618 LIFESCI
TITLE: Role of the main enzyme proteins of koji-mold Aspergillus in sedimentation of coagula in soy sauce during pasteurization.
AUTHOR: Motai, H.; Hayashi, K.; Ishiyama, T.; Sonehara, T.
CORPORATE SOURCE: Central Res. Lab., Kikkoman Corp., 399 Noda, Noda-shi, Chiba-ken 278, Japan
SOURCE: J. AGRIC. CHEM. SOC. JAP., (1983) vol. 57, no. 1, pp. 27-36
.
DOCUMENT TYPE: Journal
FILE SEGMENT: W; A; K
LANGUAGE: Japanese
SUMMARY LANGUAGE: English
AB **Alpha-amylase, acidic** and alkaline proteases derived from koji-mold Aspergillus are responsible for turbid-formation of shoyu (Japanese fermented soy sauce). The contribution to the sedimentation of coagula during the shoyu pasteurization process was investigated by measuring the amount, density and particle sizes of the coagula. These findings show that the retardation of coagulation at the final stage was not due to proteolytic action. The results indicate that **alpha -amylase** has no effect, **acidic** protease has some promoting effect and alkaline protease has a remarkable retarding effect on the sedimentation of coagula in shoyu.

L6 ANSWER 19 OF 27 MEDLINE
ACCESSION NUMBER: 79161711 MEDLINE
DOCUMENT NUMBER: 79161711 PubMed ID: 749472
TITLE: The influence of charged matrix surfaces on the thermostabilizing effect of calcium ions on immobilized fungal **alpha-amylase**.
AUTHOR: Fischer J; Ulbrich R; Schellenberger A
SOURCE: ACTA BIOLOGICA ET MEDICA GERMANICA, (1978) 37 (9) 1413-24. Journal code: 0370276. ISSN: 0001-5318.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197906
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19790611
AB The stabilizing effect of calcium ions on fungal **alpha-amylase** (EC 3.2.1.1) immobilized on a polystyrene anion exchanger (P+ amylase) was investigated and compared to the behaviour of soluble amylase. Moreover, gamma-(1,4-benzoquinone-2-yl)-aminopropyl silica-amylase (Si(n) amylase) as a conjugate with weakly basic amino groups and gamma-succinamidopropyl silica amylase (Si- amylase) as a conjugate with free carboxyl groups were applied for comparison. Depending on the calcium ion concentration the immobilized amylases showed a lower thermal stability than the soluble enzyme. The reduced stability was attributed to matrix effects in the microenvironment of the immobilized amylases and the calcium ion concentration in the carrier phase, which was changed in comparison with the external solution. Contrary to the non-measurable matrix effects in the microenvironment, altered calcium ion concentrations in the carrier phase of the polystyrene anion exchanger (P+) and gamma-succinamidopropyl silica (Si-) could be detected. With increasing calcium ion concentration a greater decrease of activity was observed for the soluble amylase than for the immobilized enzymes. The thermal stability of soluble amylase and P+ amylase was studied in dependence on pH. In the **acidic** pH-range P+ amylase indicated a higher thermal stability than the soluble enzyme in the presence of Ca2+

as well as in the absence of Ca2+. Contrary to soluble amylase the stabilizing effect of calcium ions on P+ amylase begins already at pH 3.5. Kinetic investigations for thermal inactivation were performed on soluble amylase and P+ amylase in the presence and absence of Ca2+ in the temperature range between 44--60 degrees C. Thermal inactivation proceeded by first order reactions. The inactivation rate constants kin served as a measure of thermal stability for discussing the stabilizing effect by Ca2+ depending on the temperature. The activation energies of inactivation EA were determined from the Arrhenius-plot of the inactivation rate constants.

L6 ANSWER 20 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1974:40965 HCAPLUS
DOCUMENT NUMBER: 80:40965
TITLE: Enzymic therapeutic preparation medicinal amylase
AUTHOR(S): Galich, I. P.; Tsyperovich, A. S.; Artyukh, A. G.; Kolesnik, L. A.
CORPORATE SOURCE: Inst. Biochem., Kiev, USSR
SOURCE: Farm. Zh. (Kiev) (1973), 28(5), 56-60
CODEN: FRZKAP
DOCUMENT TYPE: Journal
LANGUAGE: Ukrainian
AB A procedure was developed for obtaining a highly active "Medicinal Amylase" prepn. from **.alpha.-amylase** produced by **Aspergillus oryzae**. The procedure involved the following steps: (1) extn. of the enzymic albumin; (2) pptn. of amylase from the ext. with EtOH; (3) salting out the albumin with (NH4)2SO4; (4) dialysis against 0.005M Ca(OAc)2, and (5) lyophilization. A noncryst. product had the amylolytic activity of 5040-5400 units/g, and had similar properties to those of triply crystd. **.alpha.-amylase**. The "Medicinal Amylase" prepn. was made by mixing the product with sucrose, lactose, or starch (worst) taken in proportion 1:5. Its LD (i.p. to white mice), min. tolerable dose, and max. lethal dose were 1.2.+-.0.281, 0.2, and 3 g/kg, resp. Moreover, it was more active and more stable than the prepn. " Medical Pancreatin". Capsules stable in **acidic** medium were also designed for the prepn. It is recommended for treatment of indigestion due to pancreas insufficiency.

L6 ANSWER 21 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1972:1148 HCAPLUS
DOCUMENT NUMBER: 76:1148
TITLE: Comparative investigation of the chemical structure of **.alpha.-amylases** from microscopic fungi
AUTHOR(S): Tsiperovich, O. S.; Kastrikina, T. F.; Perevozchenko, I. I.
CORPORATE SOURCE: Inst. Biochem., Kiev, USSR
SOURCE: Ukr. Biokhim. Zh. (1971), 43(4), 441-6
CODEN: UBZHAZ
DOCUMENT TYPE: Journal
LANGUAGE: Ukrainian
AB All 3 **.alpha.-amylases** of Aspergillus flavus, A. Awamori, and A. oryzae, highly purified and obtained from the native strains, had practically the same amino acid compn., contg. considerable amts. of dicarboxylic acids and threonine. The content of **acidic** amino acids considerably exceeded the content of basic amino acids. Alanine was the N-terminal acid for all 3 enzymes. The amylases did not contain SH groups reacting directly with Na nitroprusside.

L6 ANSWER 22 OF 27 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1973:104461 BIOSIS
DOCUMENT NUMBER: BA55:4454

TITLE: A DIFFERENTIAL METHOD OF DETERMINING THE ACTIVITY OF **ACID-RESISTANT** AND NON-**ACID-RESISTANT** FUNGAL AMYLASES.

AUTHOR(S): ZHEREBTSOV N A

SOURCE: IZV VYSSH UCHEBN ZAVED PISHCH TEKHNOL, (1971) (6), 158-160. CODEN: IVUPA8. ISSN: 0579-3009.

FILE SEGMENT: BA; OLD

LANGUAGE: Unavailable

L6 ANSWER 23 OF 27 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1970:128950 HCAPLUS

DOCUMENT NUMBER: 72:128950

TITLE: Characteristics of the amylolytic complex and acid inactivation of mold .**alpha**.-**amylase**

AUTHOR(S): Feniksova, R. V.; Ermoshina, G. K.

CORPORATE SOURCE: A.N. Bakh Inst. Biochem., Moscow, USSR

SOURCE: Prikl. Biokhim. Mikrobiol. (1970), 6(1), 58-61 CODEN: PBMIAK

DOCUMENT TYPE: Journal

LANGUAGE: Russian

AB The process of acid inactivation of .**alpha**.-**amylase** of **Aspergillus oryzae** 3-9-15 in comparison with amylases of A. awamori, A. usamii and A. niger and the characteristics of the amylolytic complex of enzymes of this Aspergillus were examd. The acid inactivation of amylase was studied in culture medium that was acidified by addn. of 0.1N HCl to different pH values. .**alpha**.-**Amylase** of A. oryzae 3-9-15 can be completely inactivated during 1 hr at 30.degree. and pH 2.5-2.8, which means that the complex of amylolytic enzymes of A. oryzae 3-9-15 does not include an **acid resistant** .**alpha**.-**amylase**. However, the amylolytic complex of enzymes of black Aspergillus was shown to include an **acid-resistant** .**alpha**.-**amylase** that remained active under those conditions. Studies of the content of **acid-resistant** .**alpha**.-**amylase** in 3 strains of A. usamii, in A. awamori, and A. niger 475 demonstrated that this enzyme is produced in the greatest amt. in A. niger 475, which produces only **acid-resistant** .**alpha**.-**amylase**. Indirect evidence was derived indicating that A. oryzae 3-9-15 had no saccharifying enzyme similar to .beta.-amylase of higher plants. A possible cause of inactivation of .**alpha**.-**amylase** in acid medium was discussed.

L6 ANSWER 24 OF 27 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1970:96931 HCAPLUS

DOCUMENT NUMBER: 72:96931

TITLE: Protective action of a substrate during **acidic** and thermal inactivation of .**alpha**.-**amylases**

AUTHOR(S): Zherebtsov, N. A.; Krayushkina, E. A.

CORPORATE SOURCE: Voronezh. Tekhnol. Inst., Voronezh, USSR

SOURCE: Izv. Vyssh. Ucheb. Zaved., Pishch. Tekhnol. (1969), (6), 35-6 CODEN: IVUPA8

DOCUMENT TYPE: Journal

LANGUAGE: Russian

AB The inactivation of .**alpha**.-**amylase** from **Aspergillus oryzae** was studied in an acetate buffer with ionic strength 0.05 at pH 3-5 and at 30-70.degree. using as substrate a 3% starch soln. The protective action of the substrate during thermal activation was due to the formation of an enzyme substrate complex stabilizing the active center of the enzyme.

L6 ANSWER 25 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1969:511622 HCAPLUS
DOCUMENT NUMBER: 71:111622
TITLE: Stability of fungal .**alpha**.-**amylase** in relation to the active acidity of intermediates during commercial bread baking
AUTHOR(S): Vedernikova, E. I.; Linetskaya, G. N.; Kozhukhar, M. M.
CORPORATE SOURCE: Ukr. Nauch.-Issled. Inst. Pishch. Prom., USSR
SOURCE: Fermenty Med., Pishch. Prom. Sel. Khoz. (1968), 221-2. Editor(s): Gulyi, M. F.. Naukova Dumka: Kiev, USSR. CODEN: 21IIAZ
DOCUMENT TYPE: Conference
LANGUAGE: Russian
AB The stability of **Aspergillus oryzae** and A. awamori . **alpha**.-**amylase**, prepd. by different methods, was investigated at pH 3.2-8.0. The highest stability of A. oryzae . **alpha**.-**amylase** was at pH 5.3-8.0, and that of A. awamori at pH 5.6-7.4. At pH below 5.3 stability of A. awamori .**alpha**.-**amylase** was higher than that of A. oryzae. No difference in stability of .**alpha**.-**amylase** prepns. obtained by different methods was found at pH 5.3-8.0, but at pH below 5.3 stability of .**alpha**.-**amylase** pptd. with 70% alc. was lower than that pptd. with 55% alc. In bread baking, addn. of lactic acid bacteria to the yeast had an unfavorable effect on the stability of the . **alpha**.-**amylase**.

L6 ANSWER 26 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1963:410689 HCAPLUS
DOCUMENT NUMBER: 59:10689
ORIGINAL REFERENCE NO.: 59:1975e-g
TITLE: Cell bound .**alpha**.-**amylase** in **Aspergillus oryzae**. III. Fixation of exocellular .**alpha**.-**amylase** to mycelia
AUTHOR(S): Tonomura, Kenzo; Iwama, Katsumichi; Futai, Fusae; Tanabe, Osamu
CORPORATE SOURCE: Fermentation Res. Inst., Chiba, Japan
SOURCE: Agr. Biol. Chem. (Tokyo) (1963), 27, 128-32
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. CA 57, 14276h. Absorption of .**alpha**.-**amylase** (I) on the mycelia of Asp. oryzae was studied. The mycelia obtained in inorg. phosphate-deficient medium was incubated with crystalline .**alpha** .-**amylase** for 30 min. at definite pH, and the loss of I activity in the medium was detd. The absorption occurred in **acidic** media (pH 4-6), and the release took place in alk. media (increased with increasing pH above 6.5 to 8.0). About 90% of the absorbed I and originally mycelia-bound I was recovered from the mycelia. Expts. with S35-labeled I showed that absorbed I was more easily released from the mycelia than the originally bound I. The zone electrophoretic pattern of the once-absorbed I was identical with that of the cryst. I. The absorbed I completely withstood acid treatment (pH 4.27 for 30 min.), while the dissolved I was inactivated 54% under the same condition.

L6 ANSWER 27 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1953:26705 HCAPLUS
DOCUMENT NUMBER: 47:26705
ORIGINAL REFERENCE NO.: 47:4545g-i
TITLE: The action of the saccharifying fungi in the mash of alcohol fermentation
AUTHOR(S): Ono, Hideo; Takahara, Yoshimasa

CORPORATE SOURCE: Govt. Brewing Research Inst., Tokyo
SOURCE: Hakko Kyokai Shi (J. Fermentation Assoc.) (1950), 8, 14-18

DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB At 55.degree., **Aspergillus oryzae** shows the greatest saccharifying velocity in the pH range of 5.4-5.6, and in this pH range amylase is seldom destroyed. Below pH 5.0, 50% of the amylase, and below 4.6, 80-90% is destroyed. At 30.degree., there are 2 pH ranges of max. saccharifying power, 5.4-5.6 and 4.4-4.6. The former range is probably most favorable for .**alpha**.-**amylase**, and the latter is most favorable for .beta.-amylase. Thus, saccharification in the koji process should be carried out at 30.degree. and pH 4.4-4.6. The amylase of Amylomyces shows also the best pH ranges, at near 5.4 and 4.6. But this prepn. is lacking in .**alpha**.-**amylase**, and in the amylo process .**alpha**.-**amylase** should be supplied from other sources. The amylase of Amylomyces is scarcely destroyed in the fermentation process. The best pH values of the amylase of Aspergillus awamori are 5.0-5.2 at 55.degree. and 4.0-4.2 at 30.degree.. This amylase is not destroyed in acid soln., and the fermentation yield in the awamori process is not lowered, even when the mash becomes **acidic**.

=> d his

(FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002

L1 45382 S ALPHA (W) AMYLASE?
L2 12942 S ASPERGILLUS(W)ORYZAE
L3 1829 S L1 AND L2
L4 404497 S THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC
L5 35 S L3 AND L4
L6 27 DUP REM L5 (8 DUPLICATES REMOVED)

=> s fungamyl
L7 73 FUNGAMYL

=> s l6 and l7
L8 2 L6 AND L7

=> d 1-2 ibib ab

L8 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of **Fungamyl**-like **alpha**-**amylase**, useful for production of maltose syrups, includes mutations that improve stability against heat and **acidic** pH;
plasmid pTAKA17 expression in bacterium cell for syrup production, dough improvement, brewing and starch liquefaction
AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE: Novozymes
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]

AB A variant (A) of a **Fungamyl**-like **alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of **Fungamyl**-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L8 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:360158 HCAPLUS
DOCUMENT NUMBER: 134:363353
TITLE: **Fungamyl**-like **Aspergillus oryzae .alpha.-amylase** variants with improved thermal stability and applications to starch processes
INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan; Pedersen, Sven
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034784	A1	20010517	WO 2000-DK626	20001110

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 1999-1617 A 19991110
AB The invention relates to a variant of a parent **Fungamyl**-like fungal **.alpha.-amylase**, which exhibits improved thermal stability at **acidic** pH suitable for, e.g., starch processes. Cloning, amino acid and encoding nucleotide sequences, and mutagenesis of **.alpha.-amylase** from **Aspergillus oryzae** are provided. Construction of variant Q153S **.alpha.-amylase** (Q173S pre-. **alpha.-amylase**) from A. oryzae is disclosed.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s maltose (a)syrup or dough or brew or beer
L9 78379 MALTOSE (A) SYRUP OR DOUGH OR BREW OR BEER

=> d his

(FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002

```
L1        45382 S ALPHA (W) AMYLASE?
L2        12942 S ASPERGILLUS(W)ORYZAE
L3         1829 S L1 AND L2
L4       404497 S THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC
L5           35 S L3 AND L4
L6           27 DUP REM L5 (8 DUPLICATES REMOVED)
L7           73 S FUNGAMYL
L8            2 S L6 AND L7
L9        78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER

=> s l5 and l9
L10           2 L5 AND L9

=> d 1-2 ibib ab

L10   ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290  BIOTECHDS
TITLE:            New variant of Fungamyl-like alpha-amylase
                  , useful for production of maltose syrups
                  , includes mutations that improve stability against heat and
                  acidic pH;
                     plasmid pTAKA17 expression in bacterium cell for syrup
                     production, dough improvement, brewing and
                     starch liquefaction
AUTHOR:           Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE:  Novozymes
LOCATION:         Bagsvaerd, Denmark.
PATENT INFO:      WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO:    DK 1999-1617 10 Nov 1999
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2001-367478 [38]
AB    A variant (A) of a Fungamyl-like alpha-amylase
      (EC-3.2.1.1) is claimed.  (A) has alteration in one of the disclosed
      amino acid regions.  Each alteration is a deletion or substitution of an
      amino acid an or insertion of an amino acid downstream of a particular
      position, and (A) retains alpha-amylase activity.
      Also claimed are: DNA construct (II); recombinant expression vector
      (III); a cell (IV) transformed with the (II) or (III); composition for
      producing high maltose syrup (HMS) or alcohol;
      dough improving or brewing composition; producing (M1) of
      liquefied starch, HMS or alcohol using (A); producing (M2) variants of
      Fungamyl-like enzymes with increased thermostability; production (M3) of
      (maltose) syrup; and immobilized (A).  (A) is used
      for producing syrups, e.g. of high maltose content, or alcohol from
      starch, as dough improver for baked goods, in brewing, to
      increase fermentability of the wort, and for liquefaction of starch.
      (47pp)

L10  ANSWER 2 OF 2  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:        2001:360158  HCAPLUS
DOCUMENT NUMBER:         134:363353
TITLE:                   Fungamyl-like Aspergillus oryzae .
                         alpha.-amylase variants with
                         improved thermal stability and applications to starch
                         processes
```

INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan; Pedersen, Sven

PATENT ASSIGNEE(S): Novozymes A/S, Den.

SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034784	A1	20010517	WO 2000-DK626	20001110

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 1999-1617 A 19991110

AB The invention relates to a variant of a parent Fungamyl-like fungal **alpha.-amylase**, which exhibits improved thermal stability at **acidic** pH suitable for, e.g., starch processes. Cloning, amino acid and encoding nucleotide sequences, and mutagenesis of **.alpha.-amylase** from **Aspergillus oryzae** are provided. Construction of variant Q153S **.alpha.-amylase** (Q173S pre-**.alpha.-amylase**) from A. oryzae is disclosed.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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=> s immobili?
L11     330343 IMMOBILI?

=> d his

     (FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002
L1        45382 S ALPHA (W) AMYLASE?
L2        12942 S ASPERGILLUS(W)ORYZAE
L3         1829 S L1 AND L2
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L5           35 S L3 AND L4
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L7           73 S FUNGAMYL
L8            2 S L6 AND L7
L9        78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER
L10           2 S L5 AND L9
L11      330343 S IMMOBILI?

=> s l5 and l11
L12           5 L5 AND L11

=> d 1-5 ibib ab

L12  ANSWER 1 OF 5     MEDLINE
```

ACCESSION NUMBER: 79161711 MEDLINE
DOCUMENT NUMBER: 79161711 PubMed ID: 749472
TITLE: The influence of charged matrix surfaces on the thermostabilizing effect of calcium ions on **immobilized** fungal **alpha-amylase**
.
AUTHOR: Fischer J; Ulbrich R; Schellenberger A
SOURCE: ACTA BIOLOGICA ET MEDICA GERMANICA, (1978) 37 (9) 1413-24.
Journal code: 0370276. ISSN: 0001-5318.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197906
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19790611

AB The stabilizing effect of calcium ions on fungal **alpha-amylase** (EC 3.2.1.1) **immobilized** on a polystyrene anion exchanger (P+ amylase) was investigated and compared to the behaviour of soluble amylase. Moreover, gamma-(1,4-benzoquinone-2-yl)-aminopropyl silica-amylase (Si(n) amylase) as a conjugate with weakly basic amino groups and gamma-succinamidopropyl silica amylase (Si- amylase) as a conjugate with free carboxyl groups were applied for comparison. Depending on the calcium ion concentration the **immobilized** amylases showed a lower thermal stability than the soluble enzyme. The reduced stability was attributed to matrix effects in the microenvironment of the **immobilized** amylases and the calcium ion concentration in the carrier phase, which was changed in comparison with the external solution. Contrary to the non-measurable matrix effects in the microenvironment, altered calcium ion concentrations in the carrier phase of the polystyrene anion exchanger (P+) and gamma-succinamidopropyl silica (Si-) could be detected. With increasing calcium ion concentration a greater decrease of activity was observed for the soluble amylase than for the **immobilized** enzymes. The thermal stability of soluble amylase and P+ amylase was studied in dependence on pH. In the **acidic** pH-range P+ amylase indicated a higher thermal stability than the soluble enzyme in the presence of Ca2+ as well as in the absence of Ca2+. Contrary to soluble amylase the stabilizing effect of calcium ions on P+ amylase begins already at pH 3.5. Kinetic investigations for thermal inactivation were performed on soluble amylase and P+ amylase in the presence and absence of Ca2+ in the temperature range between 44--60 degrees C. Thermal inactivation proceeded by first order reactions. The inactivation rate constants kin served as a measure of thermal stability for discussing the stabilizing effect by Ca2+ depending on the temperature. The activation energies of inactivation EA were determined from the Arrhenius-plot of the inactivation rate constants.

L12 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1985:410715 BIOSIS
DOCUMENT NUMBER: BA80:80707
TITLE: ISOLATION OF AN AMYLASE INHIBITOR FROM SETARIA-ITALICA GRAINS BY AFFINITY CHROMATOGRAPHY ON BLUE-SEPHAROSE AND ITS CHARACTERIZATION.
AUTHOR(S): NAGARAJ R H; PATTABIRAMAN T N
CORPORATE SOURCE: DEPARTMENT OF BIOCHEMISTRY, KASTURBA MEDICAL COLLEGE, MANIPAL-576 119, INDIA.
SOURCE: J AGRIC FOOD CHEM, (1985) 33 (4), 646-650.
CODEN: JAFCAU. ISSN: 0021-8561.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB An **.alpha.-amylase** inhibitor from S. italica grains

was purified 150-fold by chromatography on Blue-Sepharose after neutralization of the acid extract and ammonium sulfate fractionation. The inhibitor was found to be homogenous by polyacrylamide gel electrophoresis [PAGE] and gel chromatography on BioGel P-30. The molecular weight was found to be 24 K. SDS[sodium dedecyl sulfate]-AGE showed that it is made up of two dissimilar polypeptides. Affinity chromatography on **immobilized** porcine pancreatic amylase and analysis showed that both the polypeptides are essential for the action of the inhibitor. The setaria inhibitor acted on human salivary amylase, human pancreatic amylase, and porcine pancreatic amylase, but had no action on Bacillus subtilis and **Aspergillus oryzae** amylases. It was labile to heat and to extreme **acidic** and alkaline conditions. Pronase, pepsin, trypsin, and .alpha.-chymotrypsin inactivated the inhibitor. Amino groups and guanido groups were found to be essential for its action.

L12 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of Fungamyl-like **alpha-amylase** , useful for production of maltose syrups, includes mutations that improve stability against heat and **acidic** pH; plasmid pTAKA17 expression in bacterium cell for syrup production, dough improvement, brewing and starch liquefaction
AUTHOR: Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE: Novozymes
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]
AB A variant (A) of a Fungamyl-like **alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity. Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of Fungamyl-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and **immobilized** (A). (A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch. (47pp)

L12 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1986-09735 BIOTECHDS
TITLE: **Immobilization** of **alpha-amylase** on polymer support; by passing **acidic alpha-amylase** solution over polyacrylonitrile column and washing out residue at same pH
PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 233589 5 Mar 1986
APPLICATION INFO: DD 1984-272358 28 Dec 1984
PRIORITY INFO: DD 1984-272358 28 Dec 1984
DOCUMENT TYPE: Patent
LANGUAGE: German

OTHER SOURCE: WPI: 1986-169840 [27]

AB For **immobilization** of **alpha-amylase** (EC-3.2.1.1) on a polymer support, the enzyme solution, of pH 2.5-4.5, is passed over polyacrylonitrile powder packed in a chromatography column. The residual constituents of the solution are then removed at the same pH. The enzyme is obtained from fungal mycelium, e.g. that of **Aspergillus oryzae**. It can be fixed to the support from non-purified solutions, without using chemical reactions. The support can be regenerated, charged repeatedly with **alpha-amylase** and re-used for enzymatic reaction. The column is used for enzymatic reactions, e.g. starch saccharification at pH 2.5-4.5. With falling activity of the column, the **alpha-amylase** is removed by washing at pH 7.5-9 and the support is cleaned. The column can be re-charged with **alpha-amylase** after washing out the alkaline solution. The **immobilized alpha-amylase** is used in the sugar industry. (2pp)

L12 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:360158 HCAPLUS

DOCUMENT NUMBER: 134:363353

TITLE: Fungamyl-like **Aspergillus oryzae** . **alpha.-amylase** variants with improved thermal stability and applications to starch processes

INVENTOR(S): Bisgard-Frantzen, Henrik; Svendsen, Allan; Pedersen, Sven

PATENT ASSIGNEE(S): Novozymes A/S, Den.

SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034784	A1	20010517	WO 2000-DK626	20001110

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 1999-1617 A 19991110

AB The invention relates to a variant of a parent Fungamyl-like fungal . **alpha.-amylase**, which exhibits improved thermal stability at **acidic** pH suitable for, e.g., starch processes. Cloning, amino acid and encoding nucleotide sequences, and mutagenesis of **.alpha.-amylase** from **Aspergillus oryzae** are provided. Construction of variant Q153S .**alpha** .-**amylase** (Q173S pre-.**alpha.-amylase**) from A. oryzae is disclosed.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> e bisgard-frantzen h/au

E1 1 BISGARD POUL/AU

E2 2 BISGARD, JAY C/AU

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E3            0 --> BISGARD-FRANTZEN H/AU
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E7            1     BISGAY L/AU
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E12          10     BISGES A D/AU

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L13           2 "BISGARDFRANTZEN H"/AU

=> d 1-2 ibib ab

L13  ANSWER 1 OF 2  SCISEARCH  COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER:     93:210039  SCISEARCH
THE GENUINE ARTICLE: KV117
TITLE:                PROTEIN ENGINEERING OF SUBTILISINS TO IMPROVE STABILITY IN
                      DETERGENT FORMULATIONS
AUTHOR:               VONDEROSTEN C (Reprint); BRANNER S; HASTRUP S; HEDEGAARD
                      L; RASMUSSEN M D; BISGARDFRANTZEN H; CARLSEN S;
                      MIKKELSEN J M
CORPORATE SOURCE:     NOVO NORD AS, DK-2880 BAGSVAERD, DENMARK (Reprint)
COUNTRY OF AUTHOR:    DENMARK
SOURCE:               JOURNAL OF BIOTECHNOLOGY, (MAR 1993) Vol. 28, No. 1, pp.
                      55-68.
                      ISSN: 0168-1656.
DOCUMENT TYPE:        Article; Journal
FILE SEGMENT:         AGRI
LANGUAGE:             ENGLISH
REFERENCE COUNT:      35
                     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB       Microbial proteases are used extensively in a large number of
      industrial processes and most importantly in detergent formulations
      facilitating the removal of proteinaceous stains. Site-directed
      mutagenesis has been employed in the construction of subtilisin variants
      with improved storage and oxidation stabilities. It is shown that in spite
      of significant structural homology between subtilisins subjected to
      protein engineering the effects of specific mutations can be quite
      different. Mutations that stabilize one subtilisin may destabilize
      another.

L13  ANSWER 2 OF 2  SCISEARCH  COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER:     89:506538  SCISEARCH
THE GENUINE ARTICLE: AR584
TITLE:                AUTOACTIVATION OF HUMAN RECOMBINANT FVII
AUTHOR:               PEDERSEN A H (Reprint); LUNDHANSEN T; BISGARDFRANTZEN
                      H; PETERSEN L C
CORPORATE SOURCE:     NOVO RES INST, DK-2880 BAGSVAERD, DENMARK
COUNTRY OF AUTHOR:    DENMARK
SOURCE:               THROMBOSIS AND HAEMOSTASIS, (1989) Vol. 62, No. 1, pp. 17.
DOCUMENT TYPE:        Conference; Journal
FILE SEGMENT:         LIFE
LANGUAGE:             ENGLISH
REFERENCE COUNT:      No References


=> e avendsen a/au
E1            1     AVENDAO REYES L/AU
E2            1     AVENDORPH, T/AU
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E3            0 --> AVENDSEN A/AU
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E5            1     AVENDT J BRIDGET/AU
E6            1     AVENDT L R/AU
E7            6     AVENDT R J/AU
E8            4     AVENDT RAYMOND J/AU
E9            1     AVENDT RAYMOND JOSEPH III/AU
E10           1     AVENDT, RAYMOND J/AU
E11           5     AVENEAU A/AU
E12           2     AVENEAU B/AU

=> e svendsen a/au
E1            1     SVENDSE F/AU
E2            6     SVENDSEN/AU
E3          286 --> SVENDSEN A/AU
E4          358     SVENDSEN A B/AU
E5          109     SVENDSEN A BAERHEIM/AU
E6            1     SVENDSEN A BARHEIM/AU
E7           12     SVENDSEN A J/AU
E8            8     SVENDSEN A K/AU
E9            4     SVENDSEN A M/AU
E10           1     SVENDSEN A M B/AU
E11           6     SVENDSEN AA P/AU
E12           3     SVENDSEN AGNER/AU

=> s e3
L14         286 "SVENDSEN A"/AU

=> d his

     (FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
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L10            2 S L5 AND L9
L11       330343 S IMMOBILI?
L12            5 S L5 AND L11
                 E BISGARD-FRANTZEN H/AU
L13            2 S E4
                 E AVENDSEN A/AU
                 E SVENDSEN A/AU
L14          286 S E3

=> s l3 and l14
L15            5 L3 AND L14

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16            3 DUP REM L15 (2 DUPLICATES REMOVED)

=> d 1-3 ibib ab

L16   ANSWER 1 OF 3  BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
```

ACCESSION NUMBER: 2001-12290 BIOTECHDS
TITLE: New variant of Fungamyl-like **alpha-amylase** , useful for production of maltose syrups, includes mutations that improve stability against heat and acidic pH; plasmid pTAKA17 expression in bacterium cell for syrup production, dough improvement, brewing and starch liquefaction
AUTHOR: Bisgard-Frantzen H; **SvendSen A**; Pedersen S
PATENT ASSIGNEE: Novozymes
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO: DK 1999-1617 10 Nov 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-367478 [38]

AB A variant (A) of a Fungamyl-like **alpha-amylase** (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed amino acid regions. Each alteration is a deletion or substitution of an amino acid an or insertion of an amino acid downstream of a particular position, and (A) retains **alpha-amylase** activity.
Also claimed are: DNA construct (II); recombinant expression vector (III); a cell (IV) transformed with the (II) or (III); composition for producing high maltose syrup (HMS) or alcohol; dough improving or brewing composition; producing (M1) of liquefied starch, HMS or alcohol using (A); producing (M2) variants of Fungamyl-like enzymes with increased thermostability; production (M3) of (maltose) syrup; and immobilized (A).
(A) is used for producing syrups, e.g. of high maltose content, or alcohol from starch, as dough improver for baked goods, in brewing, to increase fermentability of the wort, and for liquefaction of starch.
(47pp)

L16 ANSWER 2 OF 3 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 2000374774 EMBASE
TITLE: Expression and characterization of a recombinant Fusarium spp. galactose oxidase.
AUTHOR: Xu F.; Golightly E.J.; Schneider P.; Berka R.M.; Brown K.M.; Johnstone J.A.; Baker D.H.; Fuglsang C.C.; Brown S.H.; **Svendsen A.**; Klotz A.V.
CORPORATE SOURCE: F. Xu, Novo Nordisk Biotech, 1445 Drew Avenue, Davis, CA 95616, United States. fengxu@nnbt.com
SOURCE: Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology, (2000) 88/1-3 (23-32).
Refs: 16
ISSN: 0273-2289 CODEN: ABIBDL
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The Fusarium spp. (Dactylium dendroides) galactose oxidase was expressed in **Aspergillus oryzae** and Fusarium venenatum hosts.
Under the control of an A. niger .**alpha**.-**amylase** or a Fusarium trypsin promoter, high level galactose oxidase expression was achieved. The recombinant oxidase expressed in the A. oryzae host was purified and characterized. The purified enzyme had a molecular weight of 66 kDa on sodium dodecyl sulfate-polymerase gel electrophoresis (SDS-PAGE) and 0.4 mol copper atom per mole protein. The stoichiometry increased to 1.2 after a Cu saturation. Based on a peroxidase-coupled assay, the enzyme preparation showed an activity of 440 turnover per second toward D-galactose (0.1 M) at pH 7 and 20.degree.C. The enzyme had an optimal temperature of 60.degree.C at pH 6.0 and an activation free Gibbs energy

of 33 kJ/mol. A series of D-galactose derivatives was tested as the reducing substrate for the oxidase. The difference in activity was interpreted by the stereospecificity of the oxidase toward the substituents in the pyranose substrate, particularly on the C5 and the cyclic hemiacetal O sites. The recombinant oxidase could act on some galactose-containing polysaccharides, such as guar gum, but was not able to oxidize several common redox compounds that lacked a primary alcohol functional group.

L16 ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1996-12567 BIOTECHDS
TITLE: New **alpha-amylase** variants; mutant enzyme construction for improved calcium dependency, substrate binding, cleavage, pH dependent activity and thermostability; application in e.g. surfactant composition

AUTHOR: **Svendsen A**; Bisgard-Frantzen H; Borchert T V
PATENT ASSIGNEE: Novo-Nordisk
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 9623874 8 Aug 1996
APPLICATION INFO: WO 1996-DK57 5 Feb 1996
PRIORITY INFO: DK 1995-1256 10 Nov 1995; DK 1995-128 3 Feb 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1996-371424 [37]

AB A method for constructing a Termamyl-like **alpha-amylase** (TAA) mutant is new in which the variant has **alpha-amylase** (AA, EC-3.2.1.1) activity and at least one altered property as compared to the parent AA. The method involves: analyzing the structure of TAA to identify an amino acid residue or structural part which alters the property; constructing a TAA variant; and testing the variant for the property. Also claimed are: a method of constructed a variant which has decreased calcium ion dependency, altered pH dependent activity, increased thermostability and reduced ability to cleave a substrate close to the branching point. The variants can be used as surfactants or for desizing or starch liquefaction. They can also be used for the production of sweeteners and ethanol from starch. (171pp)

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=> e pedersen s/au
E1            1     PEDERSEN RONALD D/AU
E2            5     PEDERSEN RUDI/AU
E3         1236 --> PEDERSEN S/AU
E4            4     PEDERSEN S */AU
E5          530     PEDERSEN S A/AU
E6            4     PEDERSEN S A S/AU
E7            1     PEDERSEN S ANKER/AU
E8          316     PEDERSEN S B/AU
E9            1     PEDERSEN S BOEL/AU
E10          55     PEDERSEN S C/AU
E11          11     PEDERSEN S D/AU
E12         171     PEDERSEN S E/AU

=> s e3
L17        1236 "PEDERSEN S"/AU

=> d his

     (FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002
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L1         45382 S ALPHA (W) AMYLASE?
L2         12942 S ASPERGILLUS(W)ORYZAE
L3          1829 S L1 AND L2
L4        404497 S THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC
L5            35 S L3 AND L4
L6            27 DUP REM L5 (8 DUPLICATES REMOVED)
L7            73 S FUNGAMYL
L8             2 S L6 AND L7
L9         78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER
L10            2 S L5 AND L9
L11       330343 S IMMOBILI?
L12            5 S L5 AND L11
                 E BISGARD-FRANTZEN H/AU
L13            2 S E4
                 E AVENDSEN A/AU
                 E SVENDSEN A/AU
L14          286 S E3
L15            5 S L3 AND L14
L16            3 DUP REM L15 (2 DUPLICATES REMOVED)
                 E PEDERSEN S/AU
L17         1236 S E3

=> s l17 and l5
L18            1 L17 AND L5

=> d all

L18   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN    2001-12290  BIOTECHDS
TI    New variant of Fungamyl-like alpha-amylase, useful
      for production of maltose syrups, includes mutations that improve
      stability against heat and acidic pH;
         plasmid pTAKA17 expression in bacterium cell for syrup production,
         dough improvement, brewing and starch liquefaction
AU    Bisgard-Frantzen H; SvendSen A; Pedersen S
PA    Novozymes
LO    Bagsvaerd, Denmark.
PI    WO 2001034784 17 May 2001
AI    WO 2000-DK626 10 Nov 2000
PRAI  DK 1999-1617 10 Nov 1999
DT    Patent
LA    English
OS    WPI: 2001-367478 [38]
AB    A variant (A) of a Fungamyl-like alpha-amylase
      (EC-3.2.1.1) is claimed.  (A) has alteration in one of the disclosed
      amino acid regions.  Each alteration is a deletion or substitution of an
      amino acid an or insertion of an amino acid downstream of a particular
      position, and (A) retains alpha-amylase activity.
      Also claimed are: DNA construct (II); recombinant expression vector
      (III); a cell (IV) transformed with the (II) or (III); composition for
      producing high maltose syrup (HMS) or alcohol; dough improving or brewing
      composition; producing (M1) of liquefied starch, HMS or alcohol using
      (A); producing (M2) variants of Fungamyl-like enzymes with increased
      thermostability; production (M3) of (maltose) syrup; and immobilized (A).
      (A) is used for producing syrups, e.g. of high maltose content, or
      alcohol from starch, as dough improver for baked goods, in brewing, to
      increase fermentability of the wort, and for liquefaction of starch.
      (47pp)
CC    K BIOCATALYSIS; K1 Isolation and Characterization; F FOOD; F1 Food and
      Food Additives; A GENETIC ENGINEERING AND FERMENTATION; A1 Nucleic Acid
      Technology; A GENETIC ENGINEERING AND FERMENTATION; A2 Fermentation
CT    ASPERGILLUS ORYZAE RECOMBINANT FUNGAMYL-LIKE
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ALPHA-AMYLASE PREP., RANDOM MUTAGENESIS, VECTOR PLASMID PTAKA17 EXPRESSION IN BACTERIUM CELL, APPL. HIGH MALTOSE CONTENT, ALCOHOL SYRUP, DOUGH IMPROVEMENT, BREWING, STARCH LIQUEFACTION FUNGUS ENZYME EC-3.2.1.1 GENE TRANSFER STRAIN IMPROVEMENT FERMENTATION (VOL.20, NO.23)

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=> d his

     (FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002
L1        45382 S ALPHA (W) AMYLASE?
L2        12942 S ASPERGILLUS(W)ORYZAE
L3         1829 S L1 AND L2
L4       404497 S THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC
L5           35 S L3 AND L4
L6           27 DUP REM L5 (8 DUPLICATES REMOVED)
L7           73 S FUNGAMYL
L8            2 S L6 AND L7
L9        78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER
L10           2 S L5 AND L9
L11      330343 S IMMOBILI?
L12           5 S L5 AND L11
                E BISGARD-FRANTZEN H/AU
L13           2 S E4
                E AVENDSEN A/AU
                E SVENDSEN A/AU
L14         286 S E3
L15           5 S L3 AND L14
L16           3 DUP REM L15 (2 DUPLICATES REMOVED)
                E PEDERSEN S/AU
L17        1236 S E3
L18           1 S L17 AND L5

=> s l3 and l17
L19           1 L3 AND L17

=> d his

     (FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002
L1        45382 S ALPHA (W) AMYLASE?
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L5           35 S L3 AND L4
L6           27 DUP REM L5 (8 DUPLICATES REMOVED)
L7           73 S FUNGAMYL
L8            2 S L6 AND L7
L9        78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER
L10           2 S L5 AND L9
L11      330343 S IMMOBILI?
L12           5 S L5 AND L11
                E BISGARD-FRANTZEN H/AU
L13           2 S E4
                E AVENDSEN A/AU
                E SVENDSEN A/AU
L14         286 S E3
L15           5 S L3 AND L14
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L16             3 DUP REM L15 (2 DUPLICATES REMOVED)
                E PEDERSEN S/AU
L17          1236 S E3
L18             1 S L17 AND L5
L19             1 S L3 AND L17

=> s l12 or l14 or l17
L20          1524 L12 OR L14 OR L17

=> s l20 and l11
L21            29 L20 AND L11

=> dup rem l21
PROCESSING COMPLETED FOR L21
L22             20 DUP REM L21 (9 DUPLICATES REMOVED)

=> d 1-20 ibib ab

L22   ANSWER 1 OF 20  BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-12290  BIOTECHDS
TITLE:            New variant of Fungamyl-like alpha-amylase
                  , useful for production of maltose syrups, includes mutations
                  that improve stability against heat and acidic pH;
                     plasmid pTAKA17 expression in bacterium cell for syrup
                     production, dough improvement, brewing and starch
                     liquefaction
AUTHOR:           Bisgard-Frantzen H; SvendSen A; Pedersen S
PATENT ASSIGNEE:  Novozymes
LOCATION:         Bagsvaerd, Denmark.
PATENT INFO:      WO 2001034784 17 May 2001
APPLICATION INFO: WO 2000-DK626 10 Nov 2000
PRIORITY INFO:    DK 1999-1617 10 Nov 1999
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2001-367478 [38]
AB    A variant (A) of a Fungamyl-like alpha-amylase
      (EC-3.2.1.1) is claimed. (A) has alteration in one of the disclosed
      amino acid regions. Each alteration is a deletion or substitution of an
      amino acid an or insertion of an amino acid downstream of a particular
      position, and (A) retains alpha-amylase activity.
      Also claimed are: DNA construct (II); recombinant expression vector
      (III); a cell (IV) transformed with the (II) or (III); composition for
      producing high maltose syrup (HMS) or alcohol; dough improving or brewing
      composition; producing (M1) of liquefied starch, HMS or alcohol using
      (A); producing (M2) variants of Fungamyl-like enzymes with increased
      thermostability; production (M3) of (maltose) syrup; and
      immobilized (A). (A) is used for producing syrups, e.g. of high
      maltose content, or alcohol from starch, as dough improver for baked
      goods, in brewing, to increase fermentability of the wort, and for
      liquefaction of starch. (47pp)

L22  ANSWER 2 OF 20  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:        2001:360158  HCAPLUS
DOCUMENT NUMBER:         134:363353
TITLE:                   Fungamyl-like Aspergillus oryzae .
                         alpha.-amylase variants with
                         improved thermal stability and applications to starch
                         processes
INVENTOR(S):             Bisgard-Frantzen, Henrik; Svendsen, Allan; Pedersen,
                         Sven
PATENT ASSIGNEE(S):      Novozymes A/S, Den.
SOURCE:                  PCT Int. Appl., 48 pp.
```

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034784	A1	20010517	WO 2000-DK626	20001110

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 1999-1617 A 19991110

AB The invention relates to a variant of a parent Fungamyl-like fungal **alpha.-amylase**, which exhibits improved thermal stability at **acidic** pH suitable for, e.g., starch processes. Cloning, amino acid and encoding nucleotide sequences, and mutagenesis of **.alpha.-amylase** from **Aspergillus oryzae** are provided. Construction of variant Q153S **.alpha.-amylase** (Q173S pre-**.alpha.-amylase**) from A. oryzae is disclosed.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

DUPLICATE 1

L22 ANSWER 3 OF 20 MEDLINE
ACCESSION NUMBER: 2002022461 MEDLINE
DOCUMENT NUMBER: 21358032 PubMed ID: 11465505
TITLE: Reduction and alkylation of proteins in preparation of two-dimensional map analysis: why, when, and how?.
AUTHOR: Herbert B; Galvani M; Hamdan M; Olivieri E; MacCarthy J; **Pedersen S**; Righetti P G
CORPORATE SOURCE: Proteome Systems, North Ryde, Sydney, NSW, Australia.
SOURCE: ELECTROPHORESIS, (2001 Jun) 22 (10) 2046-57. Journal code: 8204476. ISSN: 0173-0835.
PUB. COUNTRY: Germany: Germany, Federal Republic of
LANGUAGE: Journal; Article; (JOURNAL ARTICLE) English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20020121 Last Updated on STN: 20020124 Entered Medline: 20020102

AB The standard procedure adopted up to the present in proteome analysis calls for just reduction prior to the isoelectric focusing/ **immobilized** pH gradient (IEF/IPG) step, followed by a second reduction/alkylation step in between the first and second dimension, in preparation for the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) step. This protocol is far from being optimal. It is here demonstrated, by matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)-mass spectrometry, that failure to reduce and alkylate proteins prior to any electrophoretic step (including the first dimension) results in a large number of spurious spots in the alkaline pH region, due to "scrambled" disulfide bridges among like and unlike chains. This series of artefactual spots comprises not only dimers, but an impressive series of oligomers (up to nonamers) in the case of simple polypeptides such as the human alpha- and beta-globin chains, which

possess only one (alpha-) or two (beta-) -SH groups. As a result, misplaced spots are to be found in the resulting two-dimensional (2-D) map, if performed with the wrong protocol. The number of such artefactual spots can be impressively large. In the case of analysis of complex samples, such as human plasma, it is additionally shown that failure to alkylate proteins results in a substantial loss of spots in the alkaline gel region, possibly due to the fact that these proteins, at their pI, regenerate their disulfide bridges with concomitant formation of macroaggregates which become entangled with and trapped within the polyacrylamide gel fibers. This strongly quenches their transfer in the subsequent SDS-PAGE step.

L22 ANSWER 4 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

ACCESSION NUMBER: 2001:554877 BIOSIS
DOCUMENT NUMBER: PREV200100554877
TITLE: High throughput multiplex genotyping using chimeric LNA (Locked Nucleic Acids)/DNA oligos **immobilized** on a polymer microchip.
AUTHOR(S): Vissing, H. (1); Nielsen, A. T. (1); Noerholm, M. (1); Mouritzen, P. (1); Hoejby, P. E. (1); **Pedersen, S. (1)**; Choleva, Y. (1); Andersen, M. S. (1); Kolberg, J. G. (1); Haagesen, K. H. (1); Kongsbak, L. (1)
CORPORATE SOURCE: (1) Bioinformatics/Genomics, Euray, EXIQON A/S, Vedbaek Denmark
SOURCE: American Journal of Human Genetics, (October, 2001) Vol. 69, No. 4 Supplement, pp. 470. print.
Meeting Info.: 51st Annual Meeting of the American Society of Human Genetics San Diego, California, USA October 12-16, 2001
ISSN: 0002-9297.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L22 ANSWER 5 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 3

ACCESSION NUMBER: 2002087426 EMBASE
TITLE: In situ studies of single enzymes and enzyme kinetics by Atomic Force Microscopy (AFM).
AUTHOR: Balashev K.; Nielsen L.K.; Callisen T.H.; **Svendsen A.**; Bjornholm T.
CORPORATE SOURCE: T. Bjornholm, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen O, Denmark. tb@symbion.ki.ku.dk
SOURCE: Probe Microscopy, (2001) 2/2 (177-185).
Refs: 72
ISSN: 1355-185X CODEN: PRMIFZ
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The time course of the degradation of lipid bilayers by Phospholipase A(2) (PLA(2)) and Humicula Lanuginosa Lipase (HLL) has been investigated using Atomic Force Microscopy (AFM). Contact mode imaging allows visualization of enzyme activity on the substrate with a high lateral resolution. By analyzing the time course of enzymatic degradation of **immobilized** lipid bilayers enzyme kinetic data are extracted and related to the lateral organization and heterogeneity of the bilayer. The first images of single HLL enzymes **immobilized** in phospholipid membranes are reported.

L22 ANSWER 6 OF 20 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1999-11105 BIOTECHDS
TITLE: Production of oligosaccharide syrups; by enzymatic catalysis using glucoamylase, glucosidase, galactosidase, xylobiase or mannosidase on sugar solution

AUTHOR: **Pedersen S**; Hendriksen H V
PATENT ASSIGNEE: Novo-Nordisk
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 9928490 10 Jun 1999
APPLICATION INFO: WO 1998-DK519 26 Nov 1998
PRIORITY INFO: DK 1997-1356 26 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-385388 [32]

AB A means of producing oligosaccharide syrups is claimed. It involves reacting a substrate with an enzyme, at 50-100 deg, to obtain a saccharide solution composed of mono-, di-, tri- and polysaccharides. The saccharide solution is then subjected to nanofiltration at 60-100 deg, to produce a syrup composed of di-, tri- and polysaccharides. The permeate of the filtration step may optionally be recycled to further enzymatic reactions. The substrate is preferably at least one monosaccharide, chosen from glucose, fructose, galactose, etc., especially a fructose and glucose mixture. It may alternatively be a liquefied starch, maltodextrin, or disaccharide solution. The enzymes are preferably **immobilized** and thermostable. They catalyze a reverse hydrolysis reaction, and are preferably glucoamylase (EC-3.2.1.3), alpha-glucosidase (EC-3.2.1.20), beta-glucosidase (EC-3.2.1.21), beta-galactosidase (EC-3.2.1.23), alpha-galactosidase (EC-3.2.1.22), xylobiase, beta-mannosidase (EC-3.2.1.25) or alpha-mannosidase (EC-3.2.1.24). Also claimed is a reactor used for this technique, and a disaccharide syrup produced by it. (39pp)

L22 ANSWER 7 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:121046 BIOSIS
DOCUMENT NUMBER: PREV200200121046
TITLE: Method of enzyme immunobilization on a particulate silica carrier for synthesis inorganic media.

AUTHOR(S): **Pedersen, S.**; Larsen, A. M.; Aasmul, P.
CORPORATE SOURCE: Gentofte Denmark
ASSIGNEE: NOVO NORDISK A-S

PATENT INFORMATION: US 5776741 July 7, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 7, 1998) Vol. 1212, No. 1, pp. 589-590.
ISSN: 0098-1133.

DOCUMENT TYPE: Patent
LANGUAGE: English

DUPLICATE 4

L22 ANSWER 8 OF 20 MEDLINE
ACCESSION NUMBER: 1998386721 MEDLINE
DOCUMENT NUMBER: 98386721 PubMed ID: 9720252
TITLE: Effect of mutations in Candida antarctica B lipase.
AUTHOR: Patkar S; Vind J; Kelstrup E; Christensen M W; **Svendsen A**; Borch K; Kirk O

CORPORATE SOURCE: Novo Nordisk A/S, Bagsvaerd, Denmark.. sap@novo.dk
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (1998 Jun) 93 (1-2) 95-101.
Journal code: 0067206. ISSN: 0009-3084.

PUB. COUNTRY: Ireland
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980918

AB Three variants of the Candida antarctica B lipase have been constructed and characterized. The variant containing the T103G mutation, which introduces the consensus sequence G-X-S-X-G found in most other known lipases, shows an increased thermostability but retains only half the specific activity of the native enzyme. Also in ester synthesis the activity is lowered but the specificity and enantioselectivity remains unchanged. The W104H mutant, in which more space is introduced into the active site, has more dramatically changed properties. Both the thermostability and the specific activity are slightly reduced but the activity and specificity in ester synthesis is highly different from the native enzyme. In general, the activity is very low and the enantioselectivity is, furthermore, highly reduced. Finally, the mutation M72L was introduced to increase the oxidation stability of the enzyme. This variant did exhibit an increased resistance towards oxidation but the thermostability was, unfortunately, also reduced.

L22 ANSWER 9 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 5
ACCESSION NUMBER: 97196461 EMBASE
DOCUMENT NUMBER: 1997196461
TITLE: Effect of mutation in non-consensus sequence Thr-X-Ser-X-Gly of Candida antarctica lipase B on lipase specificity, specific activity and thermostability.
AUTHOR: Patkar S.A.; **Svendsen A.**; Kirk O.; Clausen I.G.; Borch K.
CORPORATE SOURCE: S.A. Patkar, Noro Nordisk A/S, Noro Alle, DK-2880 Bagsvaerd, Denmark
SOURCE: Journal of Molecular Catalysis - B Enzymatic, (1997) 3/1-4 (51-54).
Refs: 18
ISSN: 1381-1177 CODEN: JMCEF8
PUBLISHER IDENT.: S 1381-1177(96)00036-7
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Non-consensus residue threonine in Candida antarctica B lipase was exchanged with glycine residue using site specific mutation. The effect of the mutation on the thermostability was investigated by measuring residual activity after heat treatment of the lipase and the mutant. A significant increase in thermostability was found for the mutant lipase. Specific activity of the mutant lipase was determined using tributyrin as substrate which showed a twofold decrease in specific activity. To investigate the effect of mutation on the specificity and activity in ester synthesis, both the mutant lipase and the native lipases were **immobilized** on a solid support. Ester synthesis using decanol as alcohol with three different fatty acids was carried out. The activity and specificity of the mutant lipase was unaltered in the ester synthesis as compared with the native lipase.

L22 ANSWER 10 OF 20 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1995-14164 BIOTECHDS
TITLE: Production of an **immobilized** enzyme with a particulate silica carrier;
lipase **immobilization** by extrusion or granulation on a silica support for use as a biocatalyst for lipid interesterification in an organic solvent system
AUTHOR: **Pedersen S**; Larsen A M; Aasmul P

PATENT ASSIGNEE: Novo-Nordisk
PATENT INFO: WO 9522606 24 Aug 1995
APPLICATION INFO: WO 1995-DK76 21 Feb 1995
PRIORITY INFO: DK 1994-207 21 Feb 1994
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1995-311314 [40]

AB A new method for producing an **immobilized** enzyme (I), active in mainly organic medium devoid of free water, involves introducing a liquid enzyme composition and particulate silica with a particle size below 100 um into a granulator or extruder and performing granulation or extrusion, respectively. The enzyme is preferably a lipase (EC-3.1.1.3) produced by culturing Humicola sp., Candida antarctica or Rhizomucor miehei. The liquid lipase composition is at least 100,000 U/g dry wt. of silica particles. The silica particles preferably have purity of over 50%, especially over 75%. The granulator is a high speed mixer or a mixer granulator. A liquid composition of a binder, preferably gelatin or polyvinylpyrrolidone, is introduced by atomization into the granulator or extruder. Granulation or extrusion preferably gives a particles size distribution of at least 90% between 50 and 2,000 um. Also new are: use of (I) for lipid interesterification involving contacting free fatty acids or fatty acid esters with (I); and use of (I) for glyceride or fatty acid ester production. The method is inexpensive and there is minimal pressure loss. (17pp)

L22 ANSWER 11 OF 20 LIFESCI COPYRIGHT 2002 CSA
ACCESSION NUMBER: 95:97022 LIFESCI
TITLE: **Immobilization** of enzymes with a cross-linking agent and a polymer containing 1-amino ethylene moieties
AUTHOR: **Pedersen, S.**; Joergensen, O.B.
CORPORATE SOURCE: Novo Nordisk A/S, Bagsvaerd (Denmark)
SOURCE: (1994) . US Patent 5,279,948; US Cl. 435/94; Int. Cl. C12P 19/24; C12N 11/08, 11/04..
DOCUMENT TYPE: Patent
FILE SEGMENT: W2
LANGUAGE: English

L22 ANSWER 12 OF 20 MEDLINE
ACCESSION NUMBER: 94183411 MEDLINE
DOCUMENT NUMBER: 94183411 PubMed ID: 8136025
TITLE: Lipases from Rhizomucor miehei and Humicola lanuginosa: modification of the lid covering the active site alters enantioselectivity.
AUTHOR: Holmquist M; Martinelle M; Berglund P; Clausen I G; Patkar S; **Svendsen A**; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: JOURNAL OF PROTEIN CHEMISTRY, (1993 Dec) 12 (6) 749-57. Journal code: 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940509
Last Updated on STN: 19990129
Entered Medline: 19940428

AB The homologous lipases from Rhizomucor miehei and Humicola lanuginosa showed approximately the same enantioselectivity when 2-methyldecanoic acid esters were used as substrates. Both lipases preferentially hydrolyzed the S-enantiomer of 1-heptyl 2-methyldecanoate (R. miehei: ES = 8.5; H. lanuginosa: ES = 10.5), but the R-enantiomer of phenyl

2-methyldecanoate (ER = 2.9). Chemical arginine specific modification of the R. miehei lipase with 1,2-cyclohexanedione resulted in a decreased enantioselectivity (ER = 2.0), only when the phenyl ester was used as a substrate. In contrast, treatment with phenylglyoxal showed a decreased enantioselectivity (ES = 2.5) only when the heptyl ester was used as a substrate. The presence of guanidine, an arginine side chain analog, decreased the enantioselectivity with the heptyl ester (ES = 1.9) and increased the enantioselectivity with the aromatic ester (ER = 4.4) as substrates. The mutation, Glu 87 Ala, in the lid of the H. lanuginosa lipase, which might decrease the electrostatic stabilization of the open-lid conformation of the lipase, resulted in 47% activity compared to the native lipase, in a tributyrin assay. The Glu 87 Ala mutant showed an increased enantioselectivity with the heptyl ester (ES = 17.4) and a decreased enantioselectivity with the phenyl ester (ER = 2.5) as substrates, compared to native lipase. The enantioselectivities of both lipases in the esterification of 2-methyldecanoic acid with 1-heptanol were unaffected by the lid modifications.

L22 ANSWER 13 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1989:192151 BIOSIS
DOCUMENT NUMBER: BR36:92600
TITLE: PROPERTIES AND APPLICATION OF SWEETZYME T A NEW **IMMOBILIZED** GLUCOSE ISOMERASE PRODUCED BY A STRAIN OF STREPTOMYCES-MURINUS.
AUTHOR(S): **PEDERSEN S**; RUGH S
CORPORATE SOURCE: NOVO INDUSTRI A/S, DK-2880 BAGSAVAERD, DEN.
SOURCE: HOLLO, J. AND D. TORLEY (ED.). BIOTECHNOLOGY AND FOOD INDUSTRY; PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM, BUDAPEST, HUNGARY, OCTOBER 5-9, 1987. XIX+707P. AKADEMIAI KIADO: BUDAPEST, HUNGARY. ILLUS, (1988) 0 (0), 267-284. ISBN: 963-05-5228-0.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L22 ANSWER 14 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
ACCESSION NUMBER: 1988:438708 BIOSIS
DOCUMENT NUMBER: BA86:90806
TITLE: A NEW **IMMOBILIZED** GLUCOSE ISOMERASE WITH HIGH PRODUCTIVITY PRODUCED BY A STRAIN OF STREPTOMYCES-MURINUS.
AUTHOR(S): JORGENSEN O B; KARLSEN L G; NIELSEN N B; **PEDERSEN S** ; RUGH S
CORPORATE SOURCE: NOVO INDUSTRI A/S, NOVO ALLE, DK-2880 BAGSVAERD, DEN.
SOURCE: STARCH STAERKE, (1988) 40 (8), 307-313. CODEN: STARDD. ISSN: 0038-9056.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The properties of a new **immobilized** glucose isomerase, produced by a selected strain of Streptomyces murinus, are described. The major advantages of the new **immobilized** enzyme are a productivity of more than 10,000 kg syrup dry substance per kg enzyme under optimal industrial conditions, increased activity and a very low syrup by-product formation. The influence of process parameters (temperature, pH and feed syrup additives) on activity and stability is discussed based on laboratory and industrial plant data.

L22 ANSWER 15 OF 20 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1990-05305 BIOTECHDS
TITLE: Properties and application of Sweetzyme T - a new **immobilized** glucose-isomerase produced by a strain of Streptomyces murinus;
comparison with Bacillus coagulans Sweetzyme Q;

application in high fructose syrup production (conference paper)

AUTHOR: **Pedersen S**; Rugh S
CORPORATE SOURCE: Novo
LOCATION: Novo Industri A/S, DK-2880 Barsvaerd, Denmark.
SOURCE: Biotechnol.Food.Ind.; (1988) 267-84
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The properties of Sweetzyme T, a glucose-isomerase (EC-5.3.1.5) produced by a mutant of Streptomyces murinus, were described and compared with those of Sweetzyme Q, the Bacillus coagulans glucose-isomerase. Nitrogen adsorption isotherms indicated that the surface area and pore volume of Sweetzyme Q are considerably larger than those of Sweetzyme T. Sweetzyme T exhibits greater stability and initial activity than Sweetzyme Q, leading to superior productivity. Typical productivity values are 8,000-11,000 kg syrup DS/kg Sweetzyme T and 3,000-6,000 kg syrup DS/kg Sweetzyme Q. The higher syrup flow rate obtained with Sweetzyme T is due to its higher initial activity and a linear activity decay mode. The life-time average flow rate may be as high as 55% of the initial flow value when Sweetzyme T is operated to 10% end-of-run activity, compared with only 39% for Sweetzyme Q. By-product formation during isomerization is minimal with Sweetzyme T. Due to its higher average activity, Sweetzyme T exhibits less flow variation in the syrup production rate than Sweetzyme Q. (9 ref)

L22 ANSWER 16 OF 20 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:495770 HCAPLUS
DOCUMENT NUMBER: 111:95770
TITLE: Properties and applications of Sweetzyme T - a new **immobilized** glucose isomerase produced by a strain of Streptomyces murinus

AUTHOR(S): **Pedersen, S.**; Rugh, S.
CORPORATE SOURCE: Novo Industri A/S, Bagsvaerd, DK-2880, Den.
SOURCE: Biotechnol. Food Ind., Proc. Int. Symp. (1988), Meeting Date 1987, 267-84. Editor(s): Hollo, J.; Torley, D. Akad. Kiado: Budapest, Hung.
CODEN: 56LIAG

DOCUMENT TYPE: Conference
LANGUAGE: English

AB The prepn. of Sweetzyme T (I) and its use in producing high-fructose corn syrup are described. S. murinus cells were disrupted, crosslinked with glutaraldehyde, and **immobilized** on a cationic flocculant. Addn. of Mg to the feed syrup activated and stabilized I. At glucose concns. >40%, the isomerization activity decreased .apprx.1% for each 1% glucose increase. Productivity in com. tests was 8000-11,000 kg syrup dry substance/kg I at an av. flow rate of 4-5 kg syrup/kg I/h. Byproduct formation of org. acids was low.

L22 ANSWER 17 OF 20 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1986-09735 BIOTECHDS
TITLE: **Immobilization** of **alpha-amylase** on polymer support; by passing **acidic alpha-amylase** solution over polyacrylonitrile column and washing out residue at same pH

PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 233589 5 Mar 1986
APPLICATION INFO: DD 1984-272358 28 Dec 1984
PRIORITY INFO: DD 1984-272358 28 Dec 1984
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1986-169840 [27]

AB For **immobilization** of **alpha-amylase** (EC-3.2.1.1) on a polymer support, the enzyme solution, of pH 2.5-4.5, is passed over polyacrylonitrile powder packed in a chromatography column. The residual constituents of the solution are then removed at the same pH. The enzyme is obtained from fungal mycelium, e.g. that of **Aspergillus oryzae**. It can be fixed to the support from non-purified solutions, without using chemical reactions. The support can be regenerated, charged repeatedly with **alpha-amylase** and re-used for enzymatic reaction. The column is used for enzymatic reactions, e.g. starch saccharification at pH 2.5-4.5. With falling activity of the column, the **alpha-amylase** is removed by washing at pH 7.5-9 and the support is cleaned. The column can be re-charged with **alpha-amylase** after washing out the alkaline solution. The **immobilized alpha-amylase** is used in the sugar industry. (2pp)

L22 ANSWER 18 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1985:410715 BIOSIS
DOCUMENT NUMBER: BA80:80707
TITLE: ISOLATION OF AN AMYLASE INHIBITOR FROM SETARIA-ITALICA GRAINS BY AFFINITY CHROMATOGRAPHY ON BLUE-SEPHAROSE AND ITS CHARACTERIZATION.
AUTHOR(S): NAGARAJ R H; PATTABIRAMAN T N
CORPORATE SOURCE: DEPARTMENT OF BIOCHEMISTRY, KASTURBA MEDICAL COLLEGE, MANIPAL-576 119, INDIA.
SOURCE: J AGRIC FOOD CHEM, (1985) 33 (4), 646-650.
CODEN: JAFCAU. ISSN: 0021-8561.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB An .**alpha**.-**amylase** inhibitor from S. italica grains was purified 150-fold by chromatography on Blue-Sepharose after neutralization of the acid extract and ammonium sulfate fractionation. The inhibitor was found to be homogenous by polyacrylamide gel electrophoresis [PAGE] and gel chromatography on BioGel P-30. The molecular weight was found to be 24 K. SDS[sodium dedecyl sulfate]-AGE showed that it is made up of two dissimilar polypeptides. Affinity chromatography on **immobilized** porcine pancreatic amylase and analysis showed that both the polypeptides are essential for the action of the inhibitor. The setaria inhibitor acted on human salivary amylase, human pancreatic amylase, and porcine pancreatic amylase, but had no action on Bacillus subtilis and **Aspergillus oryzae** amylases. It was labile to heat and to extreme **acidic** and alkaline conditions. Pronase, pepsin, trypsin, and .alpha.-chymotrypsin inactivated the inhibitor. Amino groups and guanido groups were found to be essential for its action.

L22 ANSWER 19 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 79252918 EMBASE
DOCUMENT NUMBER: 1979252918
TITLE: Traumatic dislocation of the hip in children.
AUTHOR: Jansen U.; **Pedersen S.**
CORPORATE SOURCE: Kir. Afd., Aabenraa Sygeh., Aabenraa, Denmark
SOURCE: Ugeskrift for Laeger, (1979) 141/39 (2672-2673).
CODEN: UGLAAD
COUNTRY: Denmark
DOCUMENT TYPE: Journal
FILE SEGMENT: 033 Orthopedic Surgery
019 Rehabilitation and Physical Medicine
LANGUAGE: Danish
SUMMARY LANGUAGE: English
AB Two cases of traumatic dislocation of the hip in children are reported. The hip joints involved were found to be normal clinically and

radiologically after periods of observation of two and three years, respectively. The treatment recommended consists of **immobilization** in plaster extension for 2-3 weeks followed by a period of 2-3 weeks without weight-bearing.

L22 ANSWER 20 OF 20 MEDLINE
ACCESSION NUMBER: 79161711 MEDLINE
DOCUMENT NUMBER: 79161711 PubMed ID: 749472
TITLE: The influence of charged matrix surfaces on the thermostabilizing effect of calcium ions on **immobilized** fungal **alpha-amylase**.
AUTHOR: Fischer J; Ulbrich R; Schellenberger A
SOURCE: ACTA BIOLOGICA ET MEDICA GERMANICA, (1978) 37 (9) 1413-24. Journal code: 0370276. ISSN: 0001-5318.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197906
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19790611

AB The stabilizing effect of calcium ions on fungal **alpha-amylase** (EC 3.2.1.1) **immobilized** on a polystyrene anion exchanger (P+ amylase) was investigated and compared to the behaviour of soluble amylase. Moreover, gamma-(1,4-benzoquinone-2-yl)-aminopropyl silica-amylase (Si(n) amylase) as a conjugate with weakly basic amino groups and gamma-succinamidopropyl silica amylase (Si- amylase) as a conjugate with free carboxyl groups were applied for comparison. Depending on the calcium ion concentration the **immobilized** amylases showed a lower thermal stability than the soluble enzyme. The reduced stability was attributed to matrix effects in the microenvironment of the **immobilized** amylases and the calcium ion concentration in the carrier phase, which was changed in comparison with the external solution. Contrary to the non-measurable matrix effects in the microenvironment, altered calcium ion concentrations in the carrier phase of the polystyrene anion exchanger (P+) and gamma-succinamidopropyl silica (Si-) could be detected. With increasing calcium ion concentration a greater decrease of activity was observed for the soluble amylase than for the **immobilized** enzymes. The thermal stability of soluble amylase and P+ amylase was studied in dependence on pH. In the **acidic** pH-range P+ amylase indicated a higher thermal stability than the soluble enzyme in the presence of Ca2+ as well as in the absence of Ca2+. Contrary to soluble amylase the stabilizing effect of calcium ions on P+ amylase begins already at pH 3.5. Kinetic investigations for thermal inactivation were performed on soluble amylase and P+ amylase in the presence and absence of Ca2+ in the temperature range between 44--60 degrees C. Thermal inactivation proceeded by first order reactions. The inactivation rate constants kin served as a measure of thermal stability for discussing the stabilizing effect by Ca2+ depending on the temperature. The activation energies of inactivation EA were determined from the Arrhenius-plot of the inactivation rate constants.

=> christensen t/au
CHRISTENSEN IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> e christensen t/au

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E1            1     CHRISTENSEN SZALANSKI, JAY J/AU
E2            3     CHRISTENSEN SZALANSKI, JAY J J/AU
E3         1060 --> CHRISTENSEN T/AU
E4          183     CHRISTENSEN T A/AU
E5          161     CHRISTENSEN T B/AU
E6           24     CHRISTENSEN T C/AU
E7           26     CHRISTENSEN T D/AU
E8           42     CHRISTENSEN T E/AU
E9            1     CHRISTENSEN T F/AU
E10         235     CHRISTENSEN T G/AU
E11         379     CHRISTENSEN T H/AU
E12           2     CHRISTENSEN T J/AU

=> s e3
L23         1060 "CHRISTENSEN T"/AU

=> s l23 and subtilisin
L24            1 L23 AND SUBTILISIN

=>

=> d all

L24   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN    1995-01824  BIOTECHDS
TI    Producing an enzyme e.g. a hydrolase or oxidase in increased yield;
         Fusarium oxysporum trypsin-like enzyme production by coexpression with
         metallo protease, alkaline protease or subtilisin capable of
         proenzyme or preproenzyme activation
AU    Hastrup S; Branner S; Jorgensen B R; Christensen T; Jorgensen B
      B; Shuster J R; Madden M; Moyer D L; Fuglsang C
PA    Novo-Nordisk; Novo-Nordisk-Biotech
PI    WO 9426925 24 Nov 1994
AI    WO 1994-US4932 4 May 1994
PRAI  DK 1993-522 5 May 1993
DT    Patent
LA    English
OS    WPI: 1995-006816 [01]
AB    Production of an active enzyme (I) involves culturing a cell expressing
      (I) as a proenzyme (Ia) or preproenzyme (Ib) and treating the product
      with a protease (II) (neutral metallo protease, an alkaline protease or
      subtilisin (EC-3.4.21.62)) and recovering the active (I) from the
      broth.  (II) may be added prior to and/or during fermentation and (Ia) or
      (Ib) and (II) may be encoded by a recombinant DNA sequence present in the
      cell from which (Ia) or (Ib) is expressed.  A 2nd aliquot of (II) may be
      added during culture.  (Ia) and (Ib) are less stable than (I).  (I) is
      preferably a protease, lipase (EC-3.1.1.3), amylase, cellulase
      (EC-3.2.1.4), an endo-1,4-beta-D-xylanase (EC-3.2.1.8), a
      polygalacturonase (EC-3.2.1.15), a peroxidase (EC-1.11.1.7), a laccase
      (EC-1.10.3.2) or a transglutaminase (EC-2.3.2.13), especially Fusarium
      oxysporum DSM 2672 trypsin (EC-3.4.21.4)-like protease.  (Ia) or (Ib) and
      (II) may be expressed in Bacillus, Streptomyces, Escherichia,
      Saccharomyces, Aspergillus or Fusarium spp.  Also new are a host
      expressing (II), (Ia) or (Ib) or both; DNA sequences encoding (Ia), (Ib)
      and (II); a vector; and recombinant (I).  (52pp)
CC    K BIOCATALYSIS; K1 Isolation and Characterization; A GENETIC ENGINEERING
      AND FERMENTATION; A1 Nucleic Acid Technology; K BIOCATALYSIS; K2
      Application
CT    FUSARIUM OXYSPORUM RECOMBINANT TRYPSIN-LIKE PROTEIN PREP., PROENZYME,
      PREPROENZYME ACTIVATION USING CO-EXPRESSED RECOMBINANT METALLO PROTEASE,
      ALKALINE PROTEASE, SUBTILISIN, DNA SEQUENCE, VECTOR EXPRESSION
      IN BACILLUS, STREPTOMYCES, ESCHERICHIA, SACCHAROMYCES, ASPERGILLUS,
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FUSARIUM SPP. ENZYME FUNGUS BACTERIUM EC-3.4.21.62 EC-3.4.21.4 CLONING PROTEIN SEQUENCE (VOL.14, NO.3)

=> d his

(FILE 'HOME' ENTERED AT 14:31:25 ON 27 JUN 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:31:57 ON 27 JUN 2002

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L1        45382 S ALPHA (W) AMYLASE?
L2        12942 S ASPERGILLUS(W)ORYZAE
L3         1829 S L1 AND L2
L4       404497 S THEMOSTAB? OR "ACID-RESISTANT" OR ACIDIC
L5           35 S L3 AND L4
L6           27 DUP REM L5 (8 DUPLICATES REMOVED)
L7           73 S FUNGAMYL
L8            2 S L6 AND L7
L9        78379 S MALTOSE (A)SYRUP OR DOUGH OR BREW OR BEER
L10           2 S L5 AND L9
L11      330343 S IMMOBILI?
L12           5 S L5 AND L11
                E BISGARD-FRANTZEN H/AU
L13           2 S E4
                E AVENDSEN A/AU
                E SVENDSEN A/AU
L14         286 S E3
L15           5 S L3 AND L14
L16           3 DUP REM L15 (2 DUPLICATES REMOVED)
                E PEDERSEN S/AU
L17        1236 S E3
L18           1 S L17 AND L5
L19           1 S L3 AND L17
L20        1524 S L12 OR L14 OR L17
L21          29 S L20 AND L11
L22          20 DUP REM L21 (9 DUPLICATES REMOVED)
                E CHRISTENSEN T/AU
L23        1060 S E3
L24           1 S L23 AND SUBTILISIN
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=>

	Document ID △	Issue Date	Title
1	US 20020025469 A1	20020228	Biological fuel cell and methods
2	US 20020068352 A1	20020606	Alpha-amylase variants with altered 1, 6-activity
3	US 4010258 A	19770301	Microbial amylase inhibitor and preparation thereof with the use of streptomyces diasticus var. amylostaticus
4	US 4717662 A	19880105	Thermal stabilization of alpha-amylase
5	US 5411666 A	19950502	Methods for removing biofilm from or preventing buildup thereof on surfaces in industrial water systems
6	US 6025168 A	20000215	Method for the production of isomalto-oligosaccharide rich syrups
7	US 6129788 A	20001010	Method of producing saccharide preparations
8	US 6136571 A	20001024	Method of producing saccharide preparations
9	US 6174700 B1	20010116	Purification of a polypeptide compound having a polysaccharide binding domain by affinity phase separation

	Document ID △	Issue Date	Title
10	US 6184011 B1	20010206	Method of releasing solid matrix affinity adsorbed particulates
11	US 6294281 B1	20010925	Biological fuel cell and method
12	US 6303346 B1	20011016	Method of producing saccharide preparations
13	US 6329182 B1	20011211	Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups
14	US 6391595 B1	20020521	Transferase and amylase, process for producing the enzymes, use thereof, and gene coding for the same

	Document ID △	Issue Date	Title
1	US 4676986 A	19870630	Schwanniomyces castellii strains and brewing process
2	US 4746517 A	19880524	Production of beer
3	US 5059430 A	19911022	Enzyme composition for retarding staling of baked goods
4	US 5139943 A	19920818	Processes for the recovery of microbially produced chymosin
5	US 5589207 A	19961231	Method of producing a frozen yeast dough product
6	US 5958727 A	19990928	Methods for modifying the production of a polypeptide
7	US 6165761 A	20001226	Carbohydrate oxidase and use thereof in baking
8	US 6254903 B1	20010703	Process for making baked articles that retain freshness

	Document ID △	Issue Date	Title
9	US 6323002 B1	20011127	Methods for modifying the production of a polypeptide

	Document ID △	Issue Date	Title
1	US 20020025469 A1	20020228	Biological fuel cell and methods
2	US 20020042112 A1	20020411	DNA DIAGNOSTICS BASED ON MASS SPECTROMETRY
3	US 4138292 A	19790206	Immobilized catalytically active substance and method of preparing the same
4	US 4239854 A	19801216	Enzyme-immobilization carriers and preparation thereof
5	US 4247642 A	19810127	Enzyme immobilization with pullulan gel
6	US 4388330 A	19830614	Process for the preparation of citrus juice containing beverages with improved cloud stability
7	US 4465772 A	19840814	Method for disinfecting and washing of immobilized lactase
8	US 4898781 A	19900206	Water-soluble microcapsules

	Document ID △	Issue Date	Title
9	US 5387426 A	19950207	Method of preparing reduced fat foods
10	US 5436019 A	19950725	Method of preparing reduced fat foods
11	US 6103463 A	20000815	Method of sorting a mixture of nucleic acid strands on a binary array
12	US 6150171 A	20001121	Thermostable alpha-galactosidase and methods of use
13	US 6294281 B1	20010925	Biological fuel cell and method

	Document ID △	Issue Date	Title
14	US 6322971 B1	20011127	Oligonucleotide arrays and their use for sorting, isolating, sequencing, and manipulating nucleic acids
15	US 6372472 B1	20020416	Filter media containing powered cellulose and immobilized lipase for swimming pool and spa water filteration
16	US 6391595 B1	20020521	Transferase and amylase, process for producing the enzymes, use thereof, and gene coding for the same

	Document ID △	Issue Date	Title
1	US 20020081670 A1	20020627	Starch debranching enzymes
2	US 5457045 A	19951010	Enzymes with xylanolytic activity
3	US 5753460 A	19980519	Amylase variants
4	US 6106828 A	20000822	Conjugation of polypeptides
5	US 6361989 B1	20020326	.alpha.-amylase and .alpha.-amylase variants

	Document ID △	Issue Date	Title
1	US 20020019009 A1	20020214	High throughput screening (HTS) assays
2	US 5589207 A	19961231	Method of producing a frozen yeast dough product
3	US 5817495 A	19981006	H.sub.2 O.sub.2 -stable peroxidase variants
4	US 5928381 A	19990727	Use of an .alpha.-amylase modified to improve oxidation stability in a combined desizing and bleaching process
5	US 6129788 A	20001010	Method of producing saccharide preparations
6	US 6136571 A	20001024	Method of producing saccharide preparations
7	US 6303346 B1	20011016	Method of producing saccharide preparations
8	US 6329182 B1	20011211	Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups

	Document ID	Issue Date	Pages	Title
1	US 6165761 A	20001226	30	Carbohydrate oxidase and use thereof in baking
2	US 6146865 A	20001114	20	Nucleic acids encoding polypeptides having pyranose oxidase activity
3	US 6074631 A	20000613	15	Reduction of malodour
4	US 6013452 A	20000111	43	Fungus wherein the areA, pepC and/or pepE genes have been inactivated
5	US 5965384 A	19991012	48	Methods for producing Humicola lipases in aspergillus
6	US 5874558 A	19990223	45	Nucleic acid encoding a recombinant humicola sp. lipase
7	US 5863759 A	19990126	53	Process for the production of protein products in aspergillus
8	US 5766912 A	19980616	45	Humicola lipase produced in aspergillus

	Document ID	Issue Date	Pages	Title
9	US 5702934 A	19971230	28	Processes for producing an enzyme
10	US 5536661 A	19960716	51	Process for the production of protein products in aspergillus

	L #	Hits	Search Text
1	L1	4148	alpha adj amylase$2
2	L2	10705	aspergillus
3	L3	432	l1 same l2
4	L4	132408	thermostabl$4 or "acid-resistant" or acidic
5	L5	14	l3 same l4
6	L6	76	fungamyl
7	L7	0	l5 same l6
8	L8	24533	"maltose syrup" or dough or brew or beer
9	L9	0	l5 same l8
10	L10	9	l3 same l8
11	L11	48813	immobiliz$3
12	L12	561	l4 same l11

	L #	Hits	Search Text
13	L13	1400	"aspergillus oryzae"
14	L14	16	l12 and l13
15	L15	5	bisgard.in.
16	L16	1013	pedersen.in.
17	L17	8	l3 and l16
18	L18	1538	christensen.in.
19	L19	2144	subtilisin
20	L20	16	l18 and l19
21	L21	0	1652.au
22	L22	6	muata$4
23	L23	42942	muta$4
24	L24	10	l20 and l23

	L #	Hits	Search Text
25	L25	0	christensenTERESA.in.